

THE URACHUS, ITS ANATOMY AND ASSOCIATED FASCIAE¹

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TWO TEXT FIGURES AND TWO PLATES (SEVEN FIGURES)

The literature concerning the anatomy of the urachus impresses one with the differences of opinion of various investigators. Luschka (1862), Wutz (1883) and more recently Begg ('30) have done careful anatomical dissections of many specimens and their findings are by no means in complete agreement. This situation is readily understandable in the light of the complex changes occurring in this region after birth and the resultant variability of adult specimens. This work was undertaken in an effort to increase the number of cases carefully studied and to survey the variations which might be expected to occur in this region.

The paper is based on anatomical observations upon thirty-five autopsy specimens from premature and term fetuses, one 3 year old infant, and over 100 adult cadavers.

I. THE BLADDER AND URACHUS AT BIRTH

The developmental processes leading toward the conditions seen at birth are well covered in the standard text books of embryology (Keibel and Mall, '10-'12; Arey, '40, etc.) and more detailed information on the changes in the relations of the bladder are dealt with in a paper by Disse (1892). Accordingly, our studies begin with the fetal period.

The fetal and neonatal material consisted of twenty-three premature and twelve full-term fetuses. Of the twenty-three

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prematures the youngest had a crown-rump measurement of 15.5 cm., the oldest a crown-rump measurement of 28 cm. The twelve full-term fetuses had crown-rump measurements varying from 31 cm. to 41 cm. Each specimen was carefully dissected with particular attention to the urachus and its relations with the surrounding structures.

With few exceptions the gross anatomical features of the thirty-five specimens were the same. The bladder dome was usually conical, gradually tapering into a well defined tubular urachus which, in turn, gradually became smaller in size as the umbilicus was approached. The urachus could be traced from the bladder apex to the umbilicus as a tubular structure, independent of either umbilical artery in all but two specimens which showed marked atrophy of the upper half of the urachus. As a rule the umbilical arteries appeared at either side of the bladder and converged toward the umbilical scar where they ended close to one another and to the urachus. The urachus itself was constantly supplied by a small artery which arose from the superior vesical artery and often gave off anastomotic branches of considerable size to the umbilical arteries. These anastomosing vessels have been mistaken for fibrous strands originating from the urachus and attaching it to the umbilical arteries (Begg, '30).

The musculature of the urachus appeared directly continuous with the bladder musculature and was a well developed coat in all specimens, being thickest at the bladder end and gradually thinning out as the umbilicus was approached.

A careful dissection of the urachus at its bladder end was done in all cases with particular attention to the presence or absence of a communication between the bladder cavity and the urachal lumen. Fifty per cent of specimens revealed a continuity of the two lumina. The opening of one into the other was always minute, being less than 1 mm. in diameter. We were unable to demonstrate the so-called valve of Wutz in any specimen. The urachal opening, as a rule, was on a level with the bladder mucosa and in no instance could we demonstrate this opening occurring on the prominence of a papilla.

Occasionally a small dimple was seen in the bladder mucosa at the site of the opening. In most instances, the opening was at the apex of the bladder cavity. In the remaining half of the cases, i.e. those revealing no communication with the bladder, the epithelial tube of the urachus could usually be followed through the muscular layer of the bladder to the level of the mucosa. In other specimens, however, the urachal epithelium ended in the bladder wall, being separated from the bladder mucosa by muscular tissue (fig. 5).

In the fetal and in many of the full term specimens a meso-urachus was demonstrable, the peritoneum being reflected about the urachus, umbilical arteries, and bladder dome. This peritoneal reflection joined the peritoneum of the anterior abdominal wall along the midline from the umbilicus to the symphysis pubis. Along this line of peritoneal reflection there was a definite membrane, comparable to a primary ventral mesentery, supporting the urachus and bladder (fig. 8).

II. ADULT SPECIMENS

Following our studies of the urachi of premature and full-term fetuses, we dissected minutely the urachi and associated structures of twenty adults and one 3 year old infant. Although the survey that follows is based primarily on these twenty-one specimens, our experience is much larger, inasmuch as one of us (L.Y.) has studied this region carefully in approximately 100 cadavers.

Our studies indicate that the neonatal relationship of the urachus and the umbilical arteries is maintained essentially unchanged in adults. The adult form of the urachus is determined by the relative amounts of growth and atrophy affecting it after birth. If the growth or proliferative changes present in the fetal urachus do not keep up with the growth of the abdominal wall, as one would expect in a vestigial structure, a variable amount of stretching of the urachus will result and serve to accentuate the atrophy in the area stretched. Since the extent of these changes is different in each case, the urachus exhibits considerable variation in appearance in different specimens.

We have divided the twenty-one specimens arbitrarily into four groups between which there is no sharp line of demarcation. In group I are placed the specimens which most nearly approach the fetal type of urachus and in group IV the specimens that exhibit the most marked degree of atrophy. Groups II and III are merely intermediate.

Group I. This group is comprised of seven adult specimens and the one specimen from a 3 year old infant. In these cases the urachus, although exhibiting a variable amount of atrophy and growth, could be traced from the bladder apex to the umbilicus as a cord, independent of the cord-like remains of the umbilical arteries, except for a relatively short distance near the umbilicus where the urachus and the obliterated arteries blended in a plexus of fibrous bands (fig. 3). The average distance from the bladder apex to the umbilicus was 13.3 cm. and the average urachal length was 12 cm. in the adult specimens. One specimen, exhibiting a communication between urachal lumen and bladder cavity, was also notable for its lack of atrophy and for the persistence of a definite peritoneal reflection (meso-urachus) which surrounded and supported the urachus and umbilical arteries. The specimen from the 3 year old infant resembled the adult specimens in every respect.

Group II. The distinguishing feature of this group is that the urachus after extending upward for a variable distance from the bladder apex apparently merges with the cord representing one of the umbilical arteries. It seems probable that the apparent union is due to the close approximation of two atrophic structures. There are four examples of this type in the present series. Each presents a variable amount of atrophy without much evidence of changes due to growth (fig. 4). The distance from the bladder apex to the umbilicus averaged 13 cm. and the urachal length averaged 6.4 cm. The upper end of the urachus apparently joined the right umbilical artery in three specimens and the left in one specimen. In one instance there was a communication between the epithelial canal of the urachus and the bladder cavity. One specimen presented

a mesentery-like peritoneal reflection about the umbilical arteries and urachus.

Group III. All the four specimens in this group have the same general gross structure. The umbilical arteries converge from either side of the bladder becoming adjacent to the urachus mid-way between the bladder apex and the umbilicus. From here the remains of each artery proceeds upward in rather close approximation to the urachus and to each other. In its upper half each artery shows considerable scarring and places where its remains are greatly reduced in diameter. The urachus is traceable from the bladder apex up to the region where the umbilical arteries become closely associated. Above this area the urachus has undergone so much atrophy that it can no longer be identified as an independent structure. The average distance from the umbilicus to the bladder apex was 12.7 cm. and the urachus averaged 5 cm. in length.

Group IV. This group is composed of five specimens. Here are assigned those specimens with a very short tubular urachus, exhibiting the greatest amount of regressive changes as compared with the previous groups. From the tubular portion an atrophic prolongation extends upward toward the umbilicus and becomes so small that it is lost from view as a gross structure before the umbilicus is reached. The umbilical arteries show a variable amount of atrophy but can be traced, independent of the urachus, to the umbilicus (fig. 7). There was an average distance of 15 cm. from the bladder apex to the umbilicus and the urachus averaged only 3.4 cm. in length.

In about a quarter of the twenty-one specimens in the foregoing groups the urachus presented the gross appearance of a tubular structure with muscular walls. In another quarter it appeared as a smaller cord of fibrous tissue. In the remaining cases the lower half of the urachus was a muscular tube which gradually tapered into a fibrous cord in its upper half. Where the urachus is represented by a muscular tube, a central epithelial canal is usually easily dissectable. This canal is small, being 1 mm. or less in diameter, and may pursue a straight or somewhat tortuous course in its supravescical portion as

well as in that portion found in the bladder musculature. In some cases the epithelial canal shows distinct dilatations which are often filled with epithelial debris. Gross continuity between the bladder cavity and urachal lumen was demonstrated in only two specimens of the entire series. This incidence forms a distinct contrast with that in the fetal specimens. The valve of Wutz could not be demonstrated in any of our specimens. In those cases in which no communication was found the urachal epithelium ended either in the musculature of the bladder (six specimens) or at the bladder mucosa (ten specimens). In three specimens the epithelial canal could not be found.

III. THE UMBILICAL PREVESICAL AND UMBILICAL VESICAL FASCIAE

Between the peritoneal cavity and the transversalis fascia there is an interesting fascial arrangement. In this paper we are concerned with that particular region which is bounded above by the umbilicus, below by the bladder and laterally by the peritoneal folds covering the obliterated hypogastric (umbilical) arteries. In this area are found two distinct fascial planes, one of peritoneal origin, the other representing the original connective tissue investment of the allantois and the umbilical arteries.

The fascial plane adjacent to the transversalis fascia is called the umbilical prevesical fascia (Delbet, 1895). This fascia originates from the peritoneum which in embryonic life is reflected around the superior portion of the bladder, umbilical arteries and the urachus to form a mesentery-like, supporting membrane. As development proceeds the two peritoneal cul-de-sacs, located between the visceral and parietal peritoneum of this reflection gradually become obliterated by fusion of their opposing surfaces, to form this fascial plane. This process of peritoneal obliteration is comparable with that occurring in the mesocolon and mesoduodenum. The umbilical pre-vesical fascia is triangular in shape with its base downward and its apex fused with the umbilical scar. Laterally,

this fascia blends with the thin extra-peritoneal tissue along the hypogastric folds of the peritoneum. Inferiorly the base of this triangular fascia extends behind the pubis to fuse with the fascia covering the superior surface of the levator ani muscle. In certain cases the peritoneal fusion may be incomplete laterally so that evidence of varying degrees of the meso-urachus remain in the adult. Thus from the peritoneal surface we may find portions of these cul-de-sacs remaining along the hypogastric folds of the peritoneum (plicae hypogastricae), particularly in their inferior portions at the middle inguinal fossae. If the opposing surfaces of this peritoneal reflection fail to fuse, the umbilical prevesical fascia will not be present as a triangular fascial layer but will be represented only by the attachment of the meso-urachus at the abdominal wall (fig. 8).

The umbilical vesical fascia is found between the parietal peritoneum and the umbilical prevesical fascia. It is a fascial sheath which surrounds the urachus, obliterated hypogastric arteries, bladder and prostate, and the nerves, blood vessels, and lymphatic vessels to these organs. In its supravescical portion it is triangular in shape, ending laterally as it surrounds the obliterated hypogastric arteries.

These fascial planes can be demonstrated in adult specimens by careful dissection (figs. 1 and 2). Very rarely is the umbilical prevesical fascia absent. Thus it is seen that the urachus and obliterated hypogastric arteries are separated from the peritoneum posteriorly by the posterior umbilico-vesical fascia and anteriorly from the transversalis fascia by the anterior layer of the umbilical-vesical fascia and the umbilical prevesical fascia.

Clinical significance of the fascial planes. These two fascial planes are of importance clinically in limiting different pathological processes in this region. Urachal infections, cysts, and neoplasms tend to be localized by the umbilical-vesical fascia if they have spread beyond the urachus. In such a case a tumescence is often evident in the midline between the pubis and umbilicus. An abscess arising from an acute prostatitis

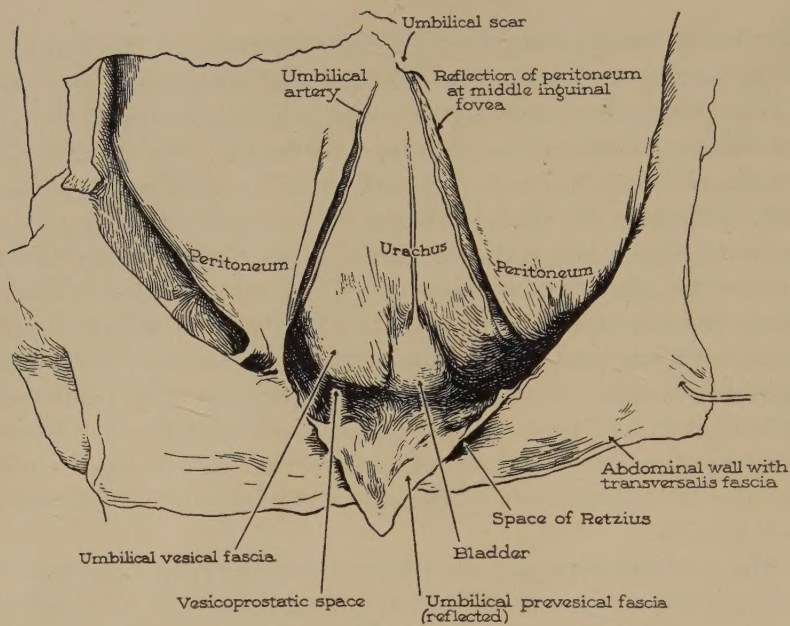


FIG. 1.

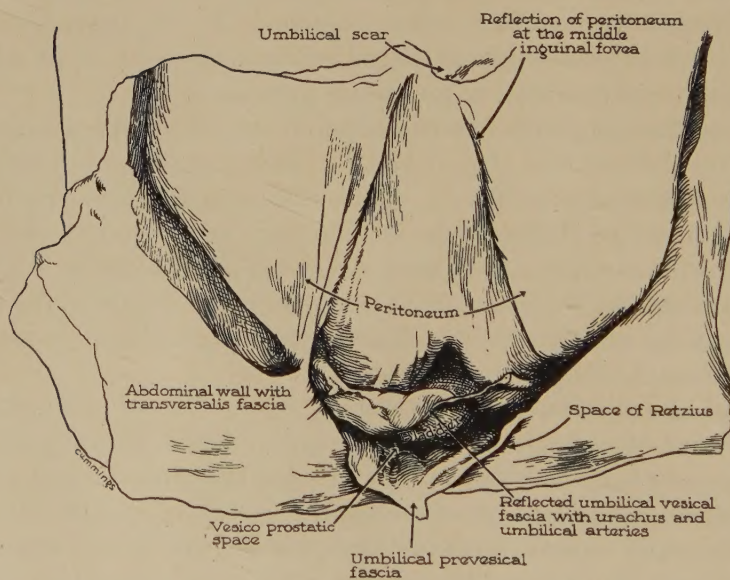


FIG. 2.

Figs. 1 and 2 Diagrammatic tracings from photographs of a lower abdominal wall dissection in an adult cadaver, showing the urachus and its associated fasciae.

may ascend in the confines of the umbilico-vesical fascia to present as a lower abdominal mass in the midline. Abscesses spreading upward from the seminal vesicles, or mesially from the broad ligament, occasionally present themselves in the lower abdomen where careful dissection will reveal that the purulent exudate is located between the peritoneum and the posterior layer of the umbilical-vesical fascia. Intra-pelvic suppuration originating from an osteomyelitis of the ilium may form an abscess in this triangular area where it will be confined between the umbilical prevesical fascia and the anterior layer of the umbilical-vesical fascia. With a thorough knowledge of these fascial layers, one can readily understand the localization of infections and other diseases of the immediate and more remote neighborhood to this triangular-shaped area between the umbilicus and pubis.

DISCUSSION

A critical survey of this series of adult specimens reveals that each urachus is likely to show minor individual variations from the general underlying plan of structure. As examples of differences in urachal development one needs only to compare the fetal type urachi of group I with the atrophic urachi of group IV. In group I, postnatal growth has played a prominent role whereas in group IV atrophy has been the dominant factor in the production of the adult form.

Our study of this region in these fetuses and adults leads us to believe that the variations encountered in the adult form of the urachus depend more upon the relative amounts of growth and atrophy affecting it after birth than upon pre-natally established differences in structure. The urachus may grow commensurately with the abdominal wall and exhibit little atrophy, maintaining essentially its fetal form. This is exemplified by the specimens of group I. In contrast the urachus may not grow to an equal degree with the abdominal wall, in which case all or a portion of it becomes stretched out and attenuated, although more or less of an umbilical connection tends to be maintained. The lower portion of the

urachus as a rule exhibits more growth and is less affected by the stretching and atrophy than the upper portion. However, it is not uncommon to find an atrophic urachus extending all the way from the bladder apex to the umbilicus.

Groups II and III are simply variations in the anatomy of the urachus in its relation to the obliterated hypogastric arteries. In both there is considerable atrophy of the upper portion of the urachus. In groups II, III, and IV there is no indication, as suggested by Begg, that the urachus has descended and dragged the obliterated ends of the arteries with it. Our studies indicate that the plexus of Luschka is formed from obliterated blood vessels which at one time anastomosed between the umbilical arteries and the urachal artery. Careful dissection of each plexus demonstrated that it was independent of the urachus and that it was intimately connected with both the umbilical arteries and the urachal artery. This plexus was a prominent finding in only three specimens. The amount of obliteration and atrophy of the umbilical arteries quite consistently reflected the amount seen in the urachus. The so-called ligamentum commune, first described by Peremescko, may be (1) the atrophic upper portion of the urachus or (2) the umbilical arteries closely associated in the midline as in group III or (3) a combination of (1) and (2).

The characteristic histology of the urachus may disappear at any level where atrophy and fibrosis are prominent.

IV. HISTOLOGY OF THE URACHUS

Histologically the average cross section of the urachus shows three layers of tissue. The innermost layer consists of a modified type of transitional epithelium having three fairly well defined layers of cells and surrounding an actual or potential central lumen. The epithelium taken altogether has a circular to oval form. Proceeding outward the next layer is oval in form and is composed of connective tissue in which a number of blood and lymph vessels are recognized. The last of the three layers is composed of bundles of involuntary muscle arranged in an irregularly circular manner. Consider-

able variation in the quantity of the muscle occurs as well as changes in form; the amount of muscle tissue is greatest near to the bladder.

Specimens suitable for histological examination have shown considerable variation. The adult urachal specimen in figure 9 averaged 3.15 mm. in diameter. Its epithelial layer was 0.3 mm. thick at the mid-point between the umbilicus and urinary bladder, and the lumen had not been obliterated. The diameter of the lumen was equal to one-third of the diameter of the surrounding epithelium. Three layers of epithelial cells were plainly recognized in the mucosa. The surrounding connective tissue, in which were a number of capillaries and some lymph vessels, was oval in form and well nourished. Surrounding this connective tissue was an oval-shaped mass of involuntary muscle bundles continued from the bladder level well up to the umbilicus. A lessened amount of muscle was present in sections near the umbilical level (also see fig. 8).

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V. SUMMARY

1. In conclusion it may be stated that the gross anatomy of the fetal and full-term urachi revealed striking similarities. In the majority the urachus and umbilical arteries converged toward the umbilicus as independent structures. The urachal artery was generally found with the urachus and anastomosed with the umbilical arteries. The bladder dome was conical and its cavity communicated with the urachus in about half of the cases.

2. The variations in the structure of the adult urachus appear to be determined by the relative extent of growth and atrophy affecting it after birth. This study has failed to confirm the so-called descent of the urachus. The urachus, on the

contrary, tends to maintain its umbilical connection as in the fetus. The epithelial canal of the urachus tends to be preserved where the urachus is represented by a muscular tube and to disappear in that part of the urachus showing considerable atrophy. A urachal canal communicated with the bladder cavity in 10% of the adult specimens.

3. The umbilical arteries and urachus are surrounded by a fascial sheath, called the umbilical vesical fascia. This fascia is separated from the transversalis fascia by the umbilical pre-vesical fascia. These fascial layers tend to localize disease of the immediate and more remote tissues to a triangular shaped area between the umbilical and pubis.

4. The urachus proper has three layers of tissue: the inner or epithelial, the submucosal and the muscular.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Figures 3, 4, 5, 6, 7 reduced one-half. Four adult specimens and one fetal specimen of urachi, umbilical arteries, and bladder apices as seen from the peritoneal surface. These figures represent the general types of urachi found in this study.

- | | |
|-----------------------|--|
| 1 Urachus. | 6 Plexus of obliterated vessels. |
| 2 Urachal epithelium. | 7 Umbilical artery. |
| 3 Bladder wall. | 8 Peritoneum. |
| 4 Bladder cavity. | 9 Urachal opening into bladder cavity. |
| 5 Umbilical scar. | 10 Fascial concentration. |

PLATE I



FIG. 3

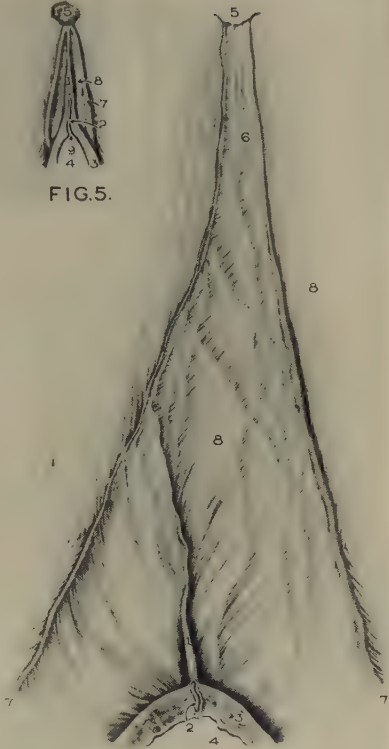


FIG. 4

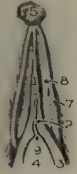


FIG. 5.

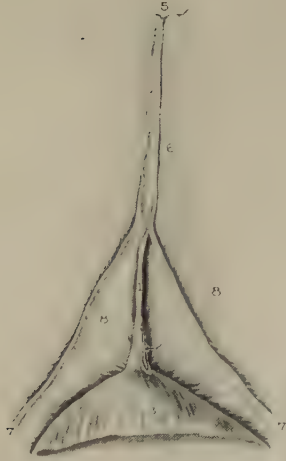


FIG. 6

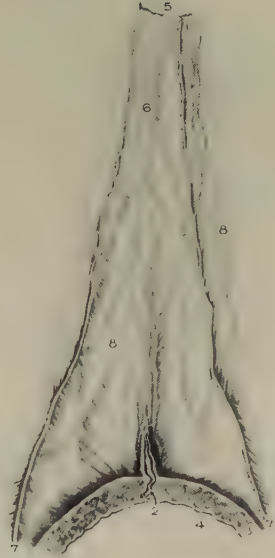


FIG. 7

PLATE 2

EXPLANATION OF FIGURES

8 Anterior part of a cross section through the entire body of a male fetus 4.4 cm. crown-rump length, magnified 150 d's. The urachus flanked by the umbilical arteries is seen in the central portion of the section. The mesourachus is shown forming the attachment of these structures to the anterior abdominal wall. When the cul-de-sac on either side of the mesourachus obliterates by fusion of the opposing peritoneal surfaces, the preumbilical-vesical fascia is formed. In the abdominal wall portions of the recti muscles and two layers of epithelial cells are formed.

9 Cross section of the urachus at its middle third from a 40-year old female. Cross size 3×1.5 mm. Epithelial layer 0.3 mm. thick. The lumen is small but patent and is surrounded by epithelium of three cells in thickness. Outside of the epithelium is the somewhat elongated oval of connective tissue and next outside of this layer is the oval-shaped mass of involuntary muscle ($\times 150$).

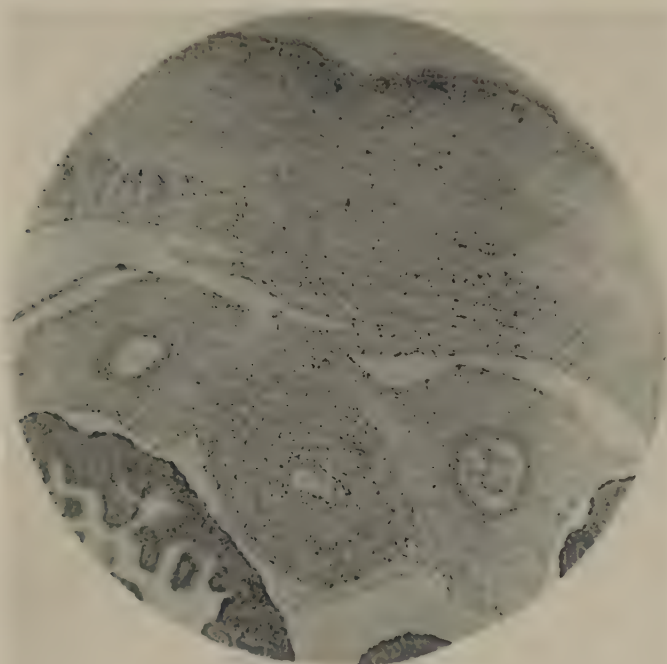


FIG.8.

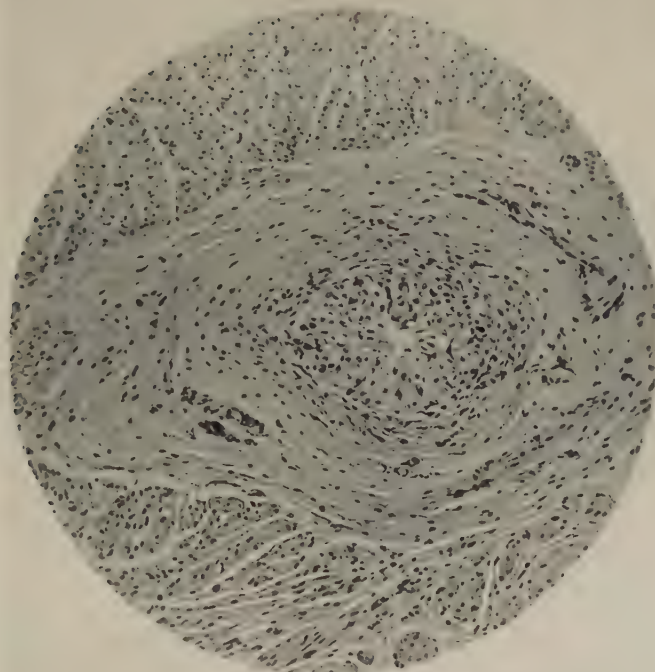


FIG.9.

THE CONE OF RENAL FASCIA IN THE ADULT WHITE MALE

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FOUR PLATES (TWENTY FIGURES)

There are few subjects in gross anatomy covering a considerable territory, where such conflicts in the current descriptions of the major features, such inaccuracy and vagueness maintain today, as that of the fasciae related to the kidney. The study of fasciae is a difficult one because they are often ill defined and present great local and individual variations. One of the greatest causes of divergent interpretations has been that collapsed panniculi of wasted bodies have been confused with fasciae. Furthermore, their pattern in the region of the kidney is influenced by seven viscera which themselves have a wide range of variation in form, size and pattern.

Toward the end of the last century, influenced no doubt by a realization of the clinical importance inherent in its close topographical relation to the kidney, the names of several outstanding anatomists became associated with its study. Among them were Toldt (1879); Sappey (1879); Zuckerkandl (1883); and Charpy (1890); as well as Tuffier (1890). Later Gerota (1895); Vecchi ('10); Southam ('23) and Vogt ('26) published accounts of rather extensive researches on this subject.

Whereas the workers just listed, in as far as they had to do with embryonic and foetal stages, usually recorded amount and character of material and frequently utilized cross sections; only the last four workers satisfied these requirements

with reference to adults and they only to a slight degree. They did not confine themselves to limited anatomical types and they used either no cross sections or only a few. The work presented in this paper is based entirely on a study of serial cross sections of selected material. It introduces the permanent recording of all cross sections by tracings and a checking of the tracings by dissecting the sections.

It is probable that a less wide range of fascial variations than described by most authors occurred in our series because of the character of the material employed. The bodies did not represent the usual chance grouping of divergent anatomical types found in the dissecting room, but formed a highly selected and homogeneous series. Bodies of medium adiposity were chosen. They were of white males varying from 45 to 60 years of age, and without senile wasting. Also bodies of individuals possessing a somewhat more robust physique than the average were selected and this probably assured the presence of unusually strong visceral and muscular fasciae. As far as could be told from external inspection, all were of the sthenic constitution. Only five of the series of sections gave evidence that there was some pathology of the connective tissue around the kidneys.

Series of transverse sections from twenty-five dextral halves of the abdomen were prepared, and twenty-five from their sinistral counterparts, making it possible to calculate the frequency of some anatomical features of the fasciae on a basis of fifty series. Each series extended from the diaphragm down into the iliac fossa. Tracings of twenty-four more series prepared later for another purpose and covering only the inferior pole and the subrenal part of the fascial cone were also used to check conclusions on the character of the anterior layer near to the mid-line. In most instances sections were one inch thick. Formalin was employed for preservation by injection and for later hardening in a tank.

Pencil tracings were prepared from the caudal face of the sections (figs. 7-20) with the exception of a few whose connective tissue characters were recorded only by notes. A series

of 404 tracings which serve as permanent records were filed for this study. The supplementary series referred to contained fifty-nine more tracings bearing on the present problem.

The term fascia is today widely used with two dissimilar meanings. We here follow the definition presented in a previous paper by the senior author ('37) while proposing a classification of gross extra-organic connective tissue structures. It follows the usage of the Basel Committee, namely that fascia is a collagenous elastic connective tissue sheet of medium or dense consistency paralleling or in contact with the surface of an organ (or organs), and adds that there is less movement between a fascia and its organ (or organs) than between a sheath and its organ.

A diagrammatic total view of renal and related fasciae are represented in figures 1 and 2 which will serve for an introductory survey of the chief features of the fasciae as found in our study. Averages of percentage frequencies were utilized in preparing many of their details. For lack of space only a few of these calculations appear in the following account. It is seen that on each side of the body only two renal fasciae occur, namely the anterior renal fascia (of Gerota) (3 of fig. 1), and the posterior renal fascia, (retro-renal fascia of Zuckerkandl), (5 of fig. 1). The anterior layer on the left side of the body is here shown with a maximal rather than an average cranial extent. The presence of two parallel fascial layers anterior to the kidneys as described by Vecchi ('10); Lewis ('04), and accepted in some anatomical texts, occurs only occasionally as a mere local duplication.

Between the renal fascia and the fibrous capsule of the kidney is a layer of perinephric panniculus adiposus ("perirenal fat body of Gerota"), (PER. of figs. 4-6). Contrary to the dominant conception today, on the average somewhat less than half of the surface of the kidneys is paralleled by renal fascia (compare right side of fig. 2). In the absence of any designation for the panniculus in contact with the kidney where renal fascia is wanting we will term it the *epinephric panniculus adiposus*.

A peculiar feature of the anterior and posterior renal fasciae taken together with a part of the psoas fascia and a small amount of the quadratus lumborum fascia is that they form a hollow figure, more cone than three sided pyramid, which caps the inferior pole of the kidney, partially enclosing that organ (fig. 2). In most of the dextral and sinistral series of sections here studied, this cone tapers to a rounded point which on the average is somewhat more blunt than shown at 9 in figures 1 and 2. The cone is of a geometrical form more suggestive of fabrication from dead rigid material than of formation from living tissues and this, we believe, is an indication of the importance of mechanical factors in its development. The long axis of the cone is more or less precisely aligned with the long axis of the kidney.

In the caudal part of the cone two of its four fascial components, the anterior and posterior renal fasciae, fuse at their lateral borders (12 of fig. 2). From their line of fusion a third fascia extends laterally in relation to the posterior aspect of the colon to terminate by uniting with the connective tissue layer of the peritoneum which forms the paracolic gutter. As we were able to find no specific designation for this fascia by the considerable number of workers who have seen it, we will term it the *lateroconal fascia*.

A more detailed description of the renal fasciae may well begin with the less complicated of the two, namely the posterior. At the outset we can confirm the claim that in most instances it is more powerfully developed than the anterior. Its cranial extent was found to be on the average approximately equal bilaterally. Its average cranial termination (fifty series) was opposite the cranial half of the body of the second lumbar vertebra with a maximal range extending from the level of the body of the twelfth thoracic vertebra to the intervertebral disc between the third and fourth lumbar vertebrae. In the caudal part of the fascial cone the medial border of the posterior layer usually terminated by fusing with the fascia which lay on the lateral face of the psoas. Occasionally it fused to the psoas fascia on the anterior face

of the muscle, or on the other hand terminated postero-laterally to the psoas by fusing with the anterior fascia of the quadratus lumborum muscle (P.R.F. of fig. 10). In most instances when traced cranially this line of fusion of the medial border of the posterior renal fascia gradually withdrew laterally on the psoas fascia till the posterior border of this muscle was reached, and then passed onto the quadratus lumborum fascia for a short distance. Frequently the line of fusion passed yet farther from the quadratus lumborum fascia to the inferior phrenic fascia in the vicinity of the lateral lumbo-costal arch.

The anterior layer of renal fascia showed great variability of extent in a cranial and in a medial direction. First, turning to the cranial limit of the sinistral layer, its greatest cranial extent was shown when it attained to the cleft bounded by the abdominal esophagus or peritoneal bare area of the stomach anteriorly, and the left crus of the diaphragm posteriorly. Such a termination was found in eight series (3 of fig. 1). In ten the fascia extended somewhat less far and came to an end upon the anterior surface of the suprarenal gland. In the remaining seven series it fell somewhat short of the suprarenal. The sinistral anterior renal fascia was always continuous medially with a connective tissue sheet related to the posterior surface of the body of the pancreas.

Sappey (1879); Gerota (1895); Lewis ('04); Vecchi ('10); and Southam ('23) claim that the anterior and posterior renal fascial layers meet cranially to the kidneys to enclose their cranial poles. Such a condition was never encountered either on the right or on the left side. To effect such an enclosure one must have recourse to large stretches of peritoneum as well as inferior phrenic fascia and this would not constitute a renal fascial enclosure.

The anterior renal fascia on the right side of the body has been claimed by Vogt ('26) to invariably terminate at the line where the transverse mesocolon attaches to the dorsal wall; or where, in the absence of such a mesentery, the colon is directly fused to the wall. We have seen that several authors

claim that it extends much farther to fuse with the posterior layer cranial to the kidney. A partial explanation for this difference of view was found in the presence of an especially delicate sheet of fascia recorded in more than half the dextral tracings as overlying the caudal part of the kidney. This delicate layer is represented by a broken line on the records (A.R.F. of fig. 16). It was usually separated from the peritoneum by a scarcely more than microscopic layer of fatty tissue, and might under surgical conditions have been interpreted as part of the peritoneum. This fascia usually extended medially beyond the hepatic face of the kidney, and approached the mid-line like typical anterior renal fascia. When traced caudally, its affinity with the renal fascia was also indicated by its invariable continuity with that fascia. In most of the series lacking the delicate layer the anterior renal fascia was limited cranially by the line of attachment of the mesocolon. The dextral anterior renal fascia including its delicate cranial part was prolonged cranially as far as the suprarenal in only three series.

The nature of the connective tissue between suprarenal and kidney in the adult is of interest as indicating whether the suprarenal would be pulled caudally by a kidney which had undergone ptosis. Sappey (1879); Vecchi ('10); and Billington ('29) are in part responsible for the view that the anterior renal fascia or one leaf of it passes between kidney and suprarenal. It was never found to take such a course in our material, and this is in agreement with Gerota (1895); and Southam ('23).

Southam ('23) and some of the earlier workers described strong fibrous bands between suprarenal and kidney. A number who have studied the character of the connective tissue between these organs in the adult describe only panniculus (Gerota, 1895; Bartlakowski, '24; Iwanow, '27; Brites, '34; and Bleicher, '31). Systematic examination of the connective tissue between the two organs in our series also revealed panniculus containing occasionally small sheets that seemed always pathological. It is of course a frequent ex-

perience in the dissecting room to find rather fine pathological fibrous sheets radiating irregularly from the kidney. This lack of normal connective tissue laminae between suprarenal and kidney combined with the strong mooring of the suprarenal by blood vessels and nerves explains the clinical observations from many sources that in ptosis the suprarenal does not move caudally with the kidney.

The medial and lateral borders of the anterior renal fascia presented a rather variable and complicated picture. It was found that an understanding of the characteristics of the lateral border of these fasciae became clarified by contrasting the part in the region of the ascending colon with that adjacent to liver or spleen. On both right and left sides of the body, where the anterior renal fascia lay opposite ascending or descending colons, the lateral borders of the anterior and posterior renal fasciae in most instances fused along a line from which sprang the *lateroconal* fascia (12 of fig. 2). The *lateroconal* layer passed laterally in the frontal plane in relation to the posterior surface of the colon to end by fusing with the connective tissue layer of the peritoneum lining the paracolic gutter (R.F. of figs. 9-14). A few sections in the region of the ascending and descending colons had no *lateroconal* fascia, the anterior and posterior renal fasciae being fused with the paracolic peritoneum along a common line or even along separate lines.

Following the lateral borders of the renal fasciae cranially, a change occurred both on the right and on the left as soon as we reached the level of the liver and of the spleen. The *lateroconal* fascia here disappeared, having decreased progressively in width as it approached this point. Cranial to the colons are the posterior peritoneal fossae in which lay a part of the right lobe of the liver on the one side of the body and the spleen on the other. Here the lateral borders of the anterior and posterior renal fasciae fused to the peritoneum just cranial to the *lateroconal* fascia usually for a short distance along a common line. When of a sufficient cranial extent, they pre-

sented yet more cranial peritoneal attachments separate from one another (1 of figs. 1 and 2).

The sinistral anterior renal fasciae of greatest cranial extent had as part of their border a line of fusion with the peritoneum where that sheet was reflected anteriorly up onto the left wall of the omental bursa which here extended from the diaphragm behind to the cranial end of the greater gastric curvature in front (3 of fig. 2).

One of the most diversely described features of the renal fasciae is their relation to the mid-line and to the corresponding contralateral fasciae. Some earlier authors believed in the continuity of both anterior and posterior renal fasciae with their counterparts across the front of the vertebral column. Many today follow Gerota (1895) in claiming such a bilateral continuity for only the anterior layer. Southam ('23), the last to make an extensive study of the total connective tissue environment of the kidney, agreed with an earlier claim by Porrier that no renal fascia crossed the mid-line. We have already seen that the posterior renal fascia never reached medially beyond its attachment to the psoas fascia, and that traced cranially its medial border withdrew yet farther laterally.

When well developed, the medial border of the anterior layer of the renal fascia presented a cranio-caudal series of four dissimilar parts comparable on the right and left sides. The caudalmost segment of the medial border on either side was characterized in most instances by a firm fusion of this border with the psoas fascia posterolateral to the ureter. Thus was formed an apex to the hollow renal cone which had no gaps in its wall (close to 9 in figs. 1 and 2, and to A.R.F. of fig. 6).

In the great majority of cases the anterior layer where fused with the psoas fascia could be split off from it. When so split, we found it continuous with an unfused medial part passing in most instances anterior to the ureter. Occasionally the fascia did not reach the ureter and occasionally the ureter was embedded in it. This medial portion of the renal fascia is

usually very thin and might readily be missed in a surgical approach. On the average the anterior layer reaches to $2\frac{1}{2}$ cm. from the mid-line. In a few sections it is traceable directly to the midsagittal sawcut and supposedly may have continued into the contralateral anterior layer.

The next segment of the border, very inconstant in occurrence on the right side of the body, extended cranially on the right until the duodenum was reached, and on the left side up to the duodenum or to the caudal border of the body of the pancreas (7 of fig. 1, and A.R.F. of fig. 4). When it approached the mid-line closely, it passed anterior to the vena cava or the aorta. Not rarely it failed to reach the ureter.

The next more cranial portion of the anterior border was most frequently in continuity with a connective tissue layer related to the posterior surface of the pars descendens duodeni on the right side of the body (4 of fig. 1 and A.R.F. of fig. 16) although in a few instances it was coterminous with a connective tissue sheet related to the lateral and anterior surfaces of this organ. The third division of the anterior layer on the left side occasionally reached a connective tissue sheet related to the posterior surface of the pars ascendens duodeni and always continued into a connective tissue layer related to the posterior surface of the body of the pancreas (11 of fig. 1 and A.R.F. of fig. 8).

The fourth and most cranial division of the medial border of the anterior layer was frequently present on the left side and rare on the right because of the lesser cranial extent of the dextral fascia. On both sides it most frequently went over into areolar tissue not far from the mid-line.

Some authors have described the ureter as perforating the anterior renal fascia. One of the most extended discussions of this happening will be found in Callander's "Surgical Anatomy," published in 1939. He states that at the point of perforation the ureter may be kinked and stenosed by a diseased and thickened renal fascia. Accordingly, systematic attention was given to the relation of the fascia to the ureter. It can be said at once that in no series from the original fifty

or from the twenty-four supplementary series did the ureter pierce the fascia. Instead, as already explained, the anterior layer in most instances passed anterior to the ureter as a thin lamina. At the level of the closed terminal part of the cone the anterior layer fused with the psoas fascia before leaving it again more medially to cross in front of the ureter. It would seem that pathological processes might be especially frequent where the ureter emerges from the cone. Young and Davis ('26) figure a position on the ureter corresponding to this region as a site for frequent adhesions which favor a kink in nephroptosis.

In keeping with the diversity of view regarding so many other features of the renal fascia a variety of arrangements are claimed for the anterior and posterior layers caudal to the kidney. Approximately the same number of workers believe that the renal fasciae meet below the kidney to enclose it as those who hold that there is no such enclosure. Gerota (1895) described a closure and Vecchi ('10) gave strong evidence for fusion below in the earlier developmental stages by study of parasagittal sections, and for the young after birth by injecting gelatin solutions into the perinephric panniculus. Neither author has furnished a definite picture of the conformation of the fascia here. In our material a complete closure below the inferior pole in the form of an apex of a cone is found in forty-five out of fifty series. In the other five series a cone was formed though the apex was incomplete.

The average cranio-caudal extent of the subrenal segment of the fascial cone was found to be 44 mm. The shortest segment was 4 mm. and the longest 107 mm. The average cranio-caudal position of the tip was 18 mm. below the summit of the iliac crest.

The apical part of the cone as seen in cross section presented several intergrading forms which in many instances were clearly such as to fit into a narrow space walled in by the psoas, the quadratus lumborum and the colon. The most variable in position of these three limiting organs is the colon, and the shape of the apex seemed frequently to be rather

strikingly affected by its location. The apices varied in cross sections from a rounded triangle (PER. of fig. 6, and AP. of fig. 14) to a blade-like form with greatest diameter in a frontal plane (AP. of fig. 15). Two subrenal segments of cones showed deep grooves on their anterior surfaces in which an obliquely running segment of a colon lay. A rare type of apex is shown in AP. of figure 17 where the psoas fascia does not form a third side. Also occasionally there is a blunter and more proximal apex utilizing leaves coming from the inner surface of the renal fasciae and this apex is followed by a more pointed distal apex.

The apex of the cone can at times be demonstrated in radiograms. Dr. Charles A. Gordon, Professor of Clinical Obstetrics and Gynecology at the Long Island College of Medicine, called the authors' attention to a radiogram taken after injection of air into the perinephric panniculus which showed an accumulation of air in a location and with a form suitable for the subrenal segment of the cone.

A secondary mechanical function of the anterior renal fascia was found to be the support of a system of veins draining some of the perinephric and paranephric panniculi. The veins we observed in the anterior renal fascia converge as they pass caudally toward the apex to two or three stems a few millimeters in diameter or even into one large vein. Near their distal end they tend to enter the perinephric panniculus and often leave the cone directly through its apex. Their terminations when noted were in the internal spermatic or in the iliolumbar vein.

CLINICAL ASPECTS OF THE CONE

The existence of a cone surrounding the more caudal part of the kidney and more particularly the lack of any gaps in the apical segment of this cone explain the observations of clinical colleagues that perinephric diseased processes do not readily pass over the iliac crest into the iliac fossa in spite of the fact that gravity favors expansion in this direction. It is to be inferred that in comparatively early stages of

perinephric abscesses they would tend to descend into the apex of the cone. Dr. Fedor L. Senger, Professor of Clinical Urology at the Long Island College of Medicine, pointed out to the authors that the reason this doubtless frequent localization is not more familiar to clinicians is that often it is only after the abscesses have undergone considerable enlargement that they can be recognized. A feature of the radiology of the perinephric pathology is the loss of sharpness of the shadow of the lateral psoas border. This is to be expected because the apex of the cone is partly formed by some fascia of the lateral psoas face. Perinephric abscesses tend to be deflected medially against the psoas fascia as they pass down into the cone. It is to be anticipated that at times they would break through the psoas fascia and develop further as psoas abscesses. Dr. Emil Goetsch, Professor of Surgery at the Long Island College of Medicine, described to the authors psoas abscesses traceable cranially to the immediate vicinity of the kidney. Supposedly they started in the perinephric or epinephric layers.

The posterior layer of renal fascia lies between much of the kidney and the subcostal, ilio-inguinal and iliohypogastric nerves. Its presence doubtless lessens the frequency of their injury in perinephric pathology. The radiology of androgenic suprarenals also has a relation to the renal fascia. The anterior layer on the right side cannot usually guide air injected into the perinephric panniculus up over the suprarenal since it was found to reach to the suprarenal in only 12% and in this region is usually extremely thin. On the left side, however, in 72% there is an anterior layer of renal fascia passing cranially over the anterior face of the suprarenal which would play a part in the movements of injected air.

A structural feature of interest for the passage of air injections and even of pathological fluids across the mid-line is the continuity of the left anterior fascial layer with a connective tissue lamina related to the posterior surface of the pancreas and the usual fusion of the right anterior layer with a connective tissue sheet related to the posterior surface

of the duodenum. The right and left anterior renal fasciae are thus both continuous with a fascial layer related to the posterior face of the duodenal-pancreatic complex described by Toldt (1879). A complete connective tissue guiding plane is thus constituted across the vertebral column in the region of duodenum and pancreas from one side to the other behind which injected air might pass. In the radiogram of a perinephric air injection previously mentioned, air had passed across the mid-line to outline the contralateral kidney.

The amount of fluid passage across the mid-line at the level of the duodenal loop depends especially on the weakness of the connective tissue lying between this plane and the posterior body wall with its vessels and nodes. Observations were not made by us upon the strength of this layer. In this connection Dr. Robert F. Barber, Professor of Clinical Surgery at the Long Island College of Medicine, communicated to the authors his experience that in surgery of the biliary passages the duodenum and pancreas seem more firmly adherent to the posterior body wall in some individuals than in others. Evidence has been given that occasionally the right and left anterior renal fasciae are continuous across the mid-line in the region caudal to duodenum and pancreas, and cranial to the apices of the renal fascial cones. There would not be any definite passageway across the mid-line behind this fascia in these instances as the connective tissue posterior to it located around the vena cava, aorta, intermesenteric nerves and lumbar lymph nodes is not especially yielding.

MECHANICAL FUNCTIONS OF THE CONE

The connective tissue about the kidney like most other sustentative tissue when examined in detail at once suggests adaptations to the local mechanical demands made upon it. Because visceral support, considered as a static condition, has been shown by Charpy (1890); Julius Tandler ('34) and others to be largely by transmission of the weight pressure of organs to subjacent portions of the body wall by way of intermediate organs and not by pull on adjacent connective

tissue structures, we can disregard the claims of some that the renal fasciae are of importance in supporting the weight of the kidney and turn our attention to the mechanics of the fascial cone in its relation to other renal movements.

Tuffier (1890) seems to have been the first to have studied extensively the movements of the kidney. He noted that in laboratory animals it undergoes expansions and contractions isochronic with the pulse beat and movements of translation isochronic with respiration. As radiology of the kidney has developed it has become clear that not only respiration, but posture affects the position of the kidney. In fact Barclay ('36) claims from radiological studies on the living that posture causes more extensive renal movements than does respiration. There is a general agreement that the chief renal displacements are along an approximately cranio-caudal path. A factor of rotation and other minor components modify the rectilinear course of the kidney. Renal movement is facilitated by the psoas acting as a track. They are also to a certain extent the result of a tendency of the kidney to swing on its pedicle. Moody and Van Nuys ('40) have considered renal movement in their recent x-ray study of renal topography on 450 individuals. They find the cranio-caudal excursion in healthy young men from posture and respiration varies from 0.0 to 8.5 cm., the most frequent condition being an excursion not less than 1 cm. and not more than 2.5 cm.

The movements of the kidney tend to be dampened down and brought to a standstill by connective tissue around it. Though the pannicular fibrous spongework around the kidney is very yielding because of its intermingling with fat cells, yet tensions develop within it which offer increasing opposition as the organ moves farther from its intermediate position. The renal pedicle will also resist certain movements.

Delicate laminae are occasionally found in the peri-, para- and epinephric panniculi which have been regarded by some as "renal ligaments" (figs. 3-6 and 8). We are convinced that most if not all of these are products of pathological processes.

It is probable that the renal fascial cone serves its chief mechanical function in resisting and limiting caudal movements of the kidney rather than in supporting renal weight. Such movements are very frequent since they occur with every inspiratory excursion of the diaphragm. They also accompany all bodily movements in which progress in a caudal direction is suddenly interrupted; as, for example, in taking a step or in landing from a jump. This down-thrust of the kidney is transmitted to the renal fasciae by way of the panniculus. The greatest pressure would, on the average, be directed downward from the inferior renal pole in the direction of the apex of the cone and these mechanical influences would become progressively less intense at more cranial levels of the cone.

The evidences of structural adaptation of the renal fascia to aid in dampening and stopping such caudal movements are numerous. As a cone capping the inferior pole of the kidney it is both formed and placed advantageously to receive these pressures. Its renal fasciae are in general stronger near the inferior renal pole. Also the cone is more complete toward the apex, the anterior layer finally fusing firmly with the psoas to assure an uninterrupted wall for more or less of the subrenal portion of the cone. Finally a primary mechanical cause for the existence of this peculiar geometrical structure seems reasonably to be found nowhere else than in the pressure emanating from the kidney. That forces originating in other organs play a minor part in its genesis requires no argument. For example, it has been seen that the colon exerts a certain moulding influence on its form.

The posterior renal fascia through its connection with the quadratus lumborum, inferior phrenic and psoas fasciae can pass on to the skeleton much of the tension set up in the cone by renal movements. Helpful in this connection is the fact that the posterior renal fascia is more powerfully developed than its anterior counterpart. There are also minor fixations of the cone by way of the lateral connections of renal fasciae with the peritoneum and by the connection of the anterior

layer with the connective tissue sheet related to the posterior surfaces of pancreas and duodenum.

CONCLUSIONS

This study of renal fasciae has to do primarily with form and topography. The most effective summary of the more common conditions of these features is embodied in figures 1 and 2. It should be noted however that the sinistral anterior renal fascia is represented in these figures with its maximal cranial extent reaching up to the abdominal esophagus and that the apices of the cones are shown as possessed of more than the average acuteness.

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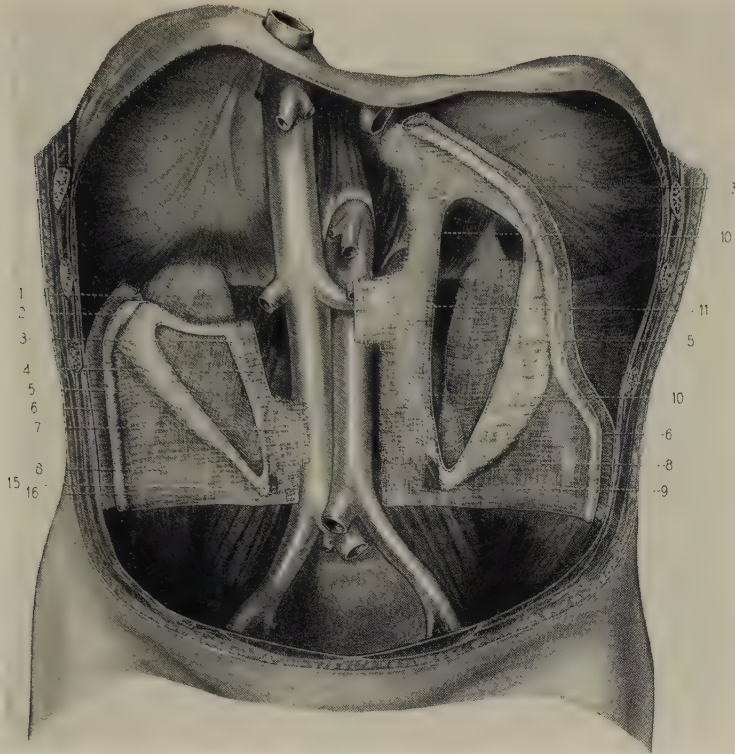
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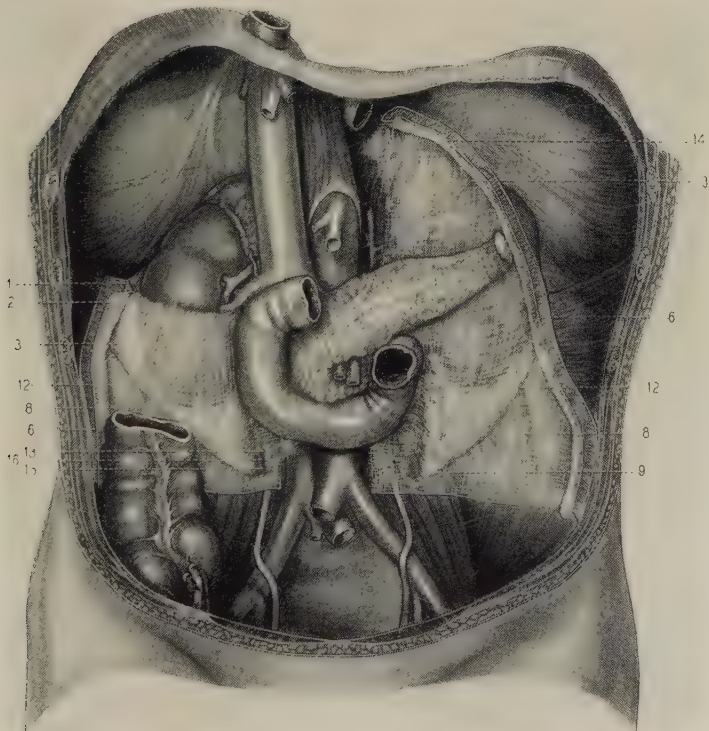
PLATE 1

EXPLANATION OF FIGURES 1 AND 2

1. Peritoneal reflection occupied chiefly by liver
2. Line of fusion of anterior renal fascia and peritoneum
3. Anterior renal fascia
4. Line of contact of anterior renal fascia and pars descendens duodeni
5. Posterior renal fascia
6. Paracolic peritoneal gutter
7. Segment of medial border of anterior renal fascia extending medially a variable distance
8. *Lateroconal* fascia
9. Apex of renal fascial cone, here represented with less than average bluntness
10. Medial border of anterior renal fascia of varying medial extent and lying caudal to pancreas, and cranial to closed part of cone
11. Anterior renal fascia continuous with connective tissue related to posterior surface of body of pancreas
12. Line of fusion of anterior renal, posterior renal and alar fasciae
13. Ureter lying behind anterior renal fascia
14. Portion of anterior renal fascia which fuses with reflection of parietal peritoneum onto sinistral wall of omental bursa cranial to lieno-renal ligament
15. Region of fusion of anterior renal and psoas fasciae. It is foreshortened because it is nearly perpendicular to the plane of the paper
16. Anterior renal fascia continued medially beyond region of fusion with psoas fascia



1



2

PLATE 2

EXPLANATION OF FIGURES

3, 4, 5 and 6 Photographs of caudal surfaces of an incomplete series of transverse sections from left side of abdomen.

A.R.F., anterior renal fascia	P.G., paracolic gutter
C., colon	P.M., psoas major muscle
K., kidney	P.R.F., posterior renal fascia
PAR., paranephric panniculus adiposus	Q.L., quadratus lumborum muscle
PER., perinephric panniculus adiposus	R.F., <i>lateroconal</i> fascia

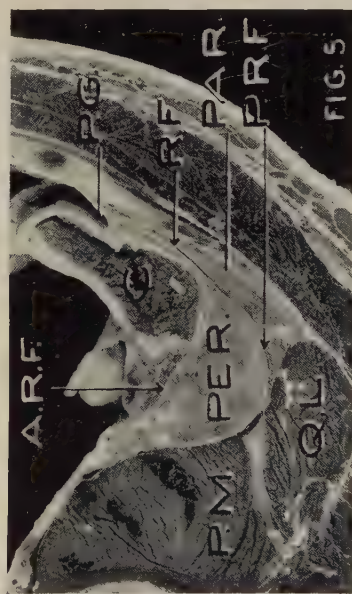
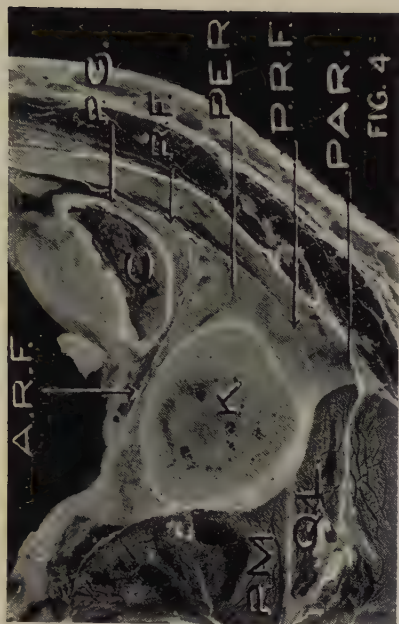


PLATE 3

EXPLANATION OF FIGURES

7, 8, 9, 10, 11, 12, 13 and 14 Tracings of caudal surfaces of a series of transverse sections from left side of abdomen.

AP., apex of cone	P.M., psoas major muscle
A.R.F., anterior renal fascia	P.R.F., posterior renal fascia
C., colon	Q.L., quadratus lumborum muscle
DI., diaphragm	R.F., <i>lateroconal</i> fascia
F., fusion of anterior renal and psoas fasciae	SB., pathological (?) connective tissue laminae
K., kidney	SP., spleen
O., continuation of anterior renal fascia medial to region of fusion	ST., stomach
P., peritoneum	SU., suprarenal gland
PAN., pancreas	U., ureter
	VE., vertebra



Fig. 7

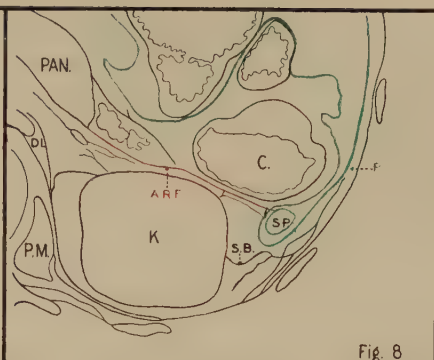


Fig. 8

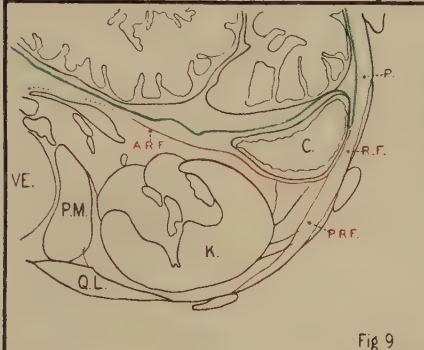


Fig. 9

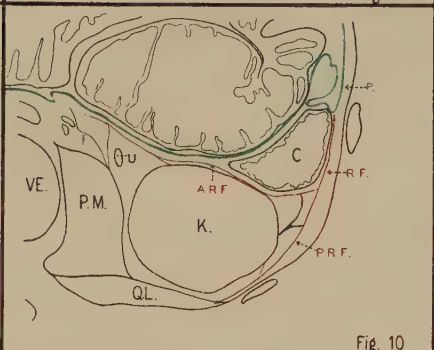


Fig. 10

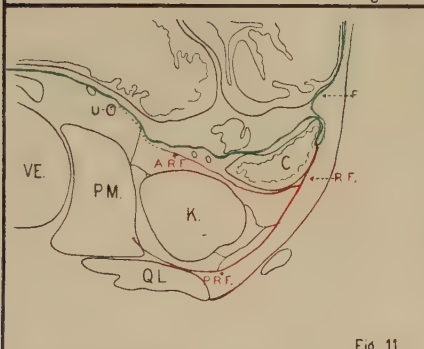


Fig. 11

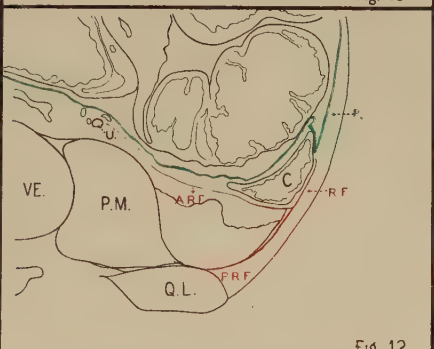


Fig. 12



Fig. 13

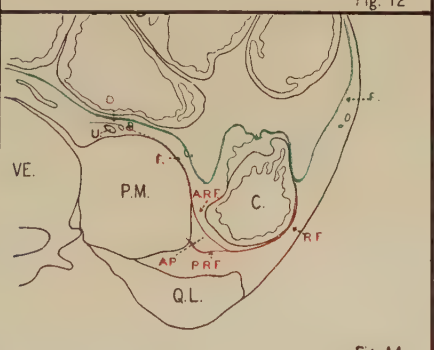


Fig. 14

PLATE 4

EXPLANATION OF FIGURES

15, 16, 17, 18, 19 and 20 Tracings of caudal surfaces of transverse sections of abdomen from various series.

A., aorta	P., peritoneum
AP., apex of cone	PAN., pancreas
A.R.F., anterior renal fascia	P.M., psoas major muscle
C., colon	P.R.F., posterior renal fascia
DI., diaphragm	Q.L., quadratus lumborum muscle
DU., duodenum	R.F., <i>lateroconal</i> fascia
I.C., iliac crest	SP., spleen
K., kidney	U., ureter
LL., liver	V., inferior vena cava
M., muscle	VE., vertebra

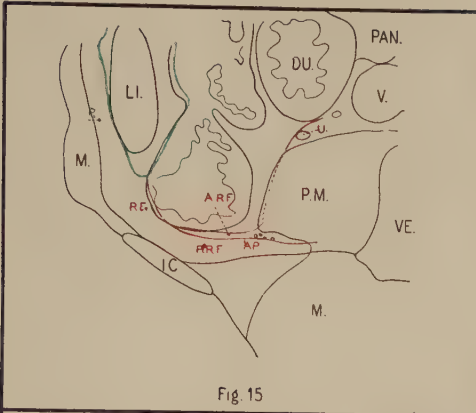


Fig. 15

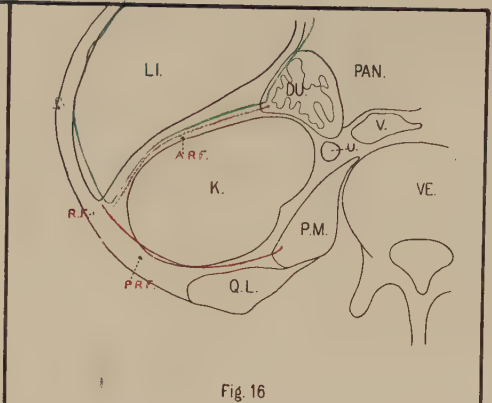


Fig. 16

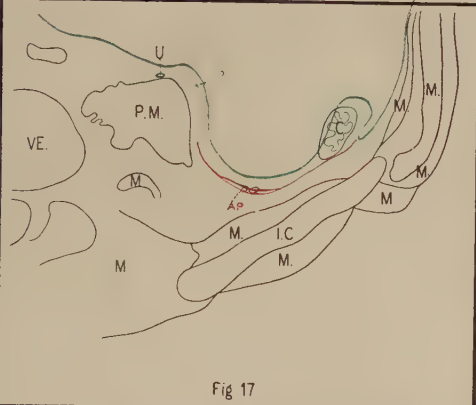


Fig. 17

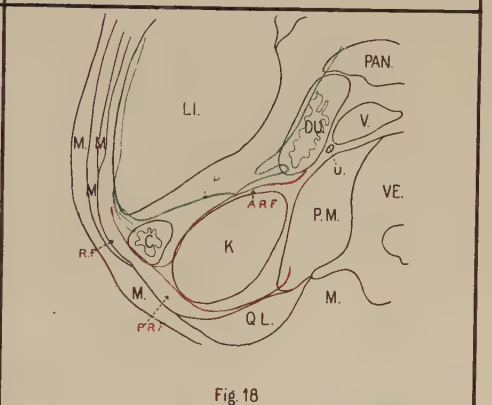


Fig. 18

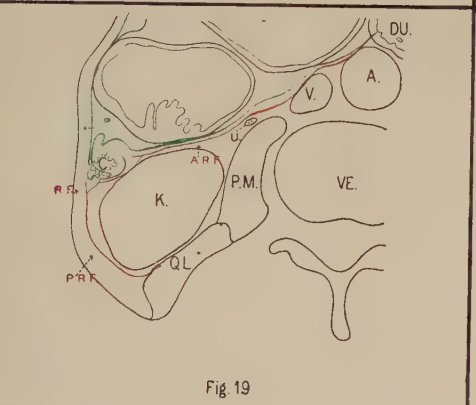


Fig. 19

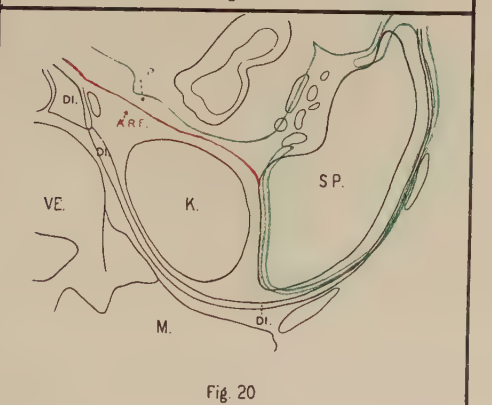


Fig. 20

EMBRYONIC DIFFERENTIATION OF OPOSSUM PROSTATE FOLLOWING CASTRATION, AND RESPONSES OF THE JUVENILE GLAND TO HORMONES ¹

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ONE TEXT FIGURE AND ONE PLATE (TWO FIGURES)

I. INTRODUCTION

In this laboratory the prostate gland of the opossum has been studied during development, and in normal adults it has been shown to react to castration and to hormonal treatments (Chase, '39). More recently the responses of the developing prostate to hormones have revealed many interesting reactions (Moore, '41). (a) In origin, the prostate tissue appears as small outgrowths from the epithelium of the urogenital sinus in males on the sixteenth or seventeenth day after birth, but the homologue in the female never appears during normal development. (b) Androgenic treatments of males stimulates precocious prostate development at all periods between the twenty-fifth and one hundredth day of pouch life, and in females its development is induced and its growth and differentiation materially advanced in comparison with development in untreated males. (c) Estrogenic treatments of developing young stimulate a marked hyperplasia and metaplastic development of the urogenital sinus epithelium, resulting in a cellular proliferation in a central direction from

¹ This investigation has been aided by grants from Armour and Company, and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago. Grateful acknowledgment is made to Dr. Dorothy Price for invaluable assistance, to Dr. Gregory Stragnell, Schering Corporation for testosterone propionate and progynon-B, and to Dr. Donald Wonder, Cutter Laboratories, for gonadin.

the basal layer rather than in a peripheral direction; in effect, therefore, the appearance and development of the prostate in males receives interference to the point of not being recognizable as prostate development. Application of estrogenic substances after formation of definite prostate cords changes these into hyperplastic cornified masses of cells that undergo dehiscence into the lumen of the urogenital canal. It becomes clear that the urogenital sinus epithelium reacts markedly different to androgens and estrogens. (d) Gonadotropic hormones applied at periods between birth and the one hundredth day evoked little to no response in prostate development, obviously because of failure of the embryonic testes to respond with secretion of male hormone.

These findings, along with consideration of many additional observations during sexual differentiation of both male and female pouch young stages, have led to the formulation of a working hypothesis which, while recognizing the capability of applied hormones to modify sexual differentiation, essentially denies a role for sex hormones (as these are known to us at present) in normal sexual differentiation of the opossum (Moore, '41).

As an extension to these previously described reactions of the developing reproductive system this report embodies an account of prostate development and differentiation (1) after removal of testes from pouch young males on day 22—5 days subsequent to prostate bud appearance—and (2) the effects of hormones on later prostate differentiation, at an age of 5 months.

II. MATERIALS AND PROCEDURE

The opossum at birth, and by its own efforts, crawls into the marsupial pouch and becomes fixed to the nipples of mammary glands; this attachment is maintained uninterruptedly for approximately 65 days and young may emerge from the pouch about day 80. They will feed from approximately the one hundredth day and have grown well when separated from the mother at the fourth month.

Successful castration has been accomplished on day 22. Since the young are largely unable to reattach to nipples after forceful separation, all operations must be done inside the mother's pouch and without undue tension on the nipple hold. A small metal plate, widely grooved in the center, has served as a support to which the young one is securely fixed, ventral side up, by adhesive tape anchorage of the four feet and tail. Sodium amytal anaesthesia of the mother successfully abolishes her movements but the young one is not anaesthetized, and asepsis is largely impossible. By day 22 of pouch life testes, attached to the sizable but degenerating mesonephros, are located in an inguinal position approachable from a mid-ventral, or bilateral inguinal, incision. The operation on individuals of approximately 30 mm. snout-rump length is done with the aid of a wide field Leitz binocular microscope. Wounds are closed, with silk thread unraveled into thirds, and covered with a thin coat of adhesive cement. Despite the fragile character of the body walls and lack of asepsis little wound infection has been experienced.

After several preliminary failures testes have been successfully removed from ten or twelve males with subsequent death of the majority, from the third to tenth postoperative day; crucial cases preserved in good condition provide the material for this section of the paper.

Responses of the juvenile, or late, prostate stage of differentiation have been studied on eight males treated for 2-week periods with sacrifice at about 5 months of age; females of similar age have likewise received treatments and will be reported elsewhere. Testosterone propionate, estradiol and gonadin were employed and at autopsy the entire reproductive tract was removed by dissection.

III. OBSERVATIONS

A. Prostate differentiation following castration

Despite late fixation of a few young found dead in the pouch, or cage, the material was sufficiently good to demonstrate clearly that prostate development continued after early

removal of the testes. Since crucial cases of young removed in good condition from the nipple for fixation are available, and since no deviations have been found from the general trend of differentiation, detailed attention in the present report will be restricted to the oldest postoperative case.

A litter of ten pouch young was discovered on the morning of April 5, 1940, which had been delivered since the previous morning and were probably under 12 hours of age. Different members of the litter were selectively removed from the pouch for preservation until on April 26th (22 days in the pouch) three males and one female remained. On this date two males were successfully castrated by a bilateral inguinal approach. On May 11th (thirty-seventh day in pouch—fifteenth day after castration) one male was removed from the nipple and preserved, after measurements, in Bouin's fluid. May 24th (day 50 in the pouch—day 28 of castration) the second operated male, together with the remaining normal male and female, were preserved. These were prepared in serial section of the reproductive tract and the operated male (series 159) will be described in detail and compared with the normal brother (series 160) removed from the same pouch.

The 28-day castrated male and its untreated brother gave respectively a snout-rump length of 75 mm. and 77 mm.; head length 26 mm. and 26.5 mm.; fresh weight 12.4 gm. and 13.0 gm. Growth and general development of the castrate, therefore, compared favorably with the normal brother; no infection had been seen at any time.

The prostate gland of the castrate was present throughout 250 serial sections (10μ) while that of the untreated control was present throughout 265 sections. Figure 2B is a cross section through urogenital sinus and prostate of the castrate male, figure 2 C a comparable section through the untreated normal, and figure 2 A a section of the urogenital sinus and prostate of a 24-day normal male (2 days older than the operated male on the day of castration).

It will be readily evident from a comparison of these figures—photomicrographs at the same magnification—that

prostate gland growth and development continues after testis removal about the stage of prostate appearance, and that 28 days after castration the prostate compares very favorably indeed with the development found in an unoperated control. In an attempt to express concretely the relative amount of development in the two cases, sections of the prostate were projected and traced on paper at a magnification of 160 times—all sections of the 24-day prostate being projected, whereas in the operated and normal 50-day specimens each third section was used. A planimeter was employed to record relative amounts of prostate tissue in the three young males; only the actual epithelial outgrowths from the urogenital sinus epithelium were considered. As a check on planimeter readings all outlines of prostate tissue were cut out and the total number of pieces weighed.

Planimeter readings, as well as the paper cut-outs, agreed in showing that the prostate of the castrate male was 3500% greater than the normal prostate gland of the 24-day normal male, which represents the amount present in the castrate on day 22. Similar determinations employed for the 50-day normal male showed that by planimeter readings this normal had increased 5000% over the 24-day stage whereas paper cut-outs showed this increase to be 5300%. Comparisons of the castrate male with its unoperated brother by planimeter readings showed it to contain 71.7% of the normal prostate, and paper cut-outs indicated 66.5% of the normal prostate mass.

It is thus realized that despite removal of the testes on day 22, or 5 to 6 days after appearance of the first primordium of this gland, prostate growth and differentiation proceed in an essentially normal manner and with a rate of development only a little below that in a normal male; the severity of the operation on the delicate young individual, and the entire lack of testes have been of little hindrance to development in this accessory organ of reproduction.

The prostate gland in a 15-day castrated male removed from the nipple in the same pouch (ser. 153; castration period

days 22-37), as well as the prostate in an individual from another pouch after 23 days of castration (ser. 162; days 23-46 — found dead in pouch, but fixation and staining excellent) agree in demonstrating that the development of this gland is but slightly retarded, if at all, following removal of the developing testes; neither of these two have been compared quantitatively with glands in normal males of similar ages.

B. Prostate responses to hormone during late development stages

The prostate gland of the opossum does not attain a functional development until approximately 1 year of age. Animals born in February were sacrificed 1 day after the last of twelve daily hormone treatments in July at an age of approximately 5 months. At this time (see fig. 3 A) the normal prostate is characterized by branching cord outgrowths of cells that retain their connections with the urogenital sinus epithelium. No canalization of the cords has yet appeared. The responsiveness of this developing tissue was studied after treatments with testosterone propionate, estradiol and the gonadotropic activity from pregnant mare serum.

Gross outlines of the urogenital sinus and prostate after treatment, in comparison with the untreated control, can be seen from figure 1 while the histological responses are shown by photomicrographs in figure 3.

The treatment of juvenile individuals with gonadin (twelve daily treatments with 20 rat units) leads to a marked increase in total size of urogenital sinus and prostate (fig. 1), as does treatments with testosterone propionate (twelve daily treatments with 2.0 mg.) and estradiol (twelve treatments with progynon-B 20 rat units). Fresh weight of the prostate (average of two animals) gives values for the untreated control, 1008 mg.; gonadin treated, 6067 mg.; t-propionate treated, 8190 mg. and estradiol treated, 7160 mg. Whereas gross weight increases follow all hormone treatments, the effects on dif-

ferentiation of prostate tissue are revealed in histological section (fig. 3).

These figures will make it readily apparent that gonadin treatments were effective in stimulating development of the prostate. The central ends of the prostate cords have developed wide open lumina, and canalization has occurred over approximately three-fourths the total length of peripherally directed cords. It is also evident that stimulation is the same

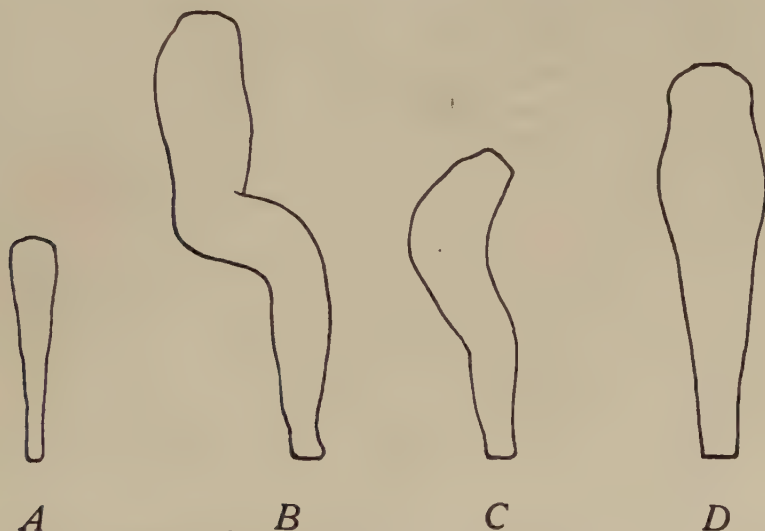


Fig. 1 Outline drawings of prostate area of urogenital sinus from 5-month-old opossums. Original $\times 2$. A, normal. B, twelve daily injections, 2.0 mg. t-prop. C, twelve daily treatments with gonadin, 20 rat units. D, twelve daily treatments with progynon-B, 20 rat units.

in quality, but less in quantity, than that occurring after testosterone treatment (fig. 3 C). Two milligrams t-propionate daily treatment for the same period has led to much greater prostate differentiation and the gland is entirely similar to that in the young puberal adult, although smaller than in large adult males. Canalization has extended over the entire length of the prostate cords and higher magnifications reveal typical secreting cells characteristic of mature adults. As was clearly pointed out by Mrs. Chase ('39) the gland shows

different types of cells making up the secreting portion that are roughly segregated into three longitudinal areas but in this juvenile group the areas are not sharply demarcated from each other.

The responses to estrogenic treatment, while involving a great increase in total mass of the urogenital sinus-prostate portion, are very different histologically from those produced by androgens. In comparing figures 3 A and D (also 1 A and D) one realizes that great cellular proliferation has followed the introduction of progynon-B; this has involved all portions of the prostate cords. The cords have been increased in total length, making them much more tortuous, and the duct portion or central ends of the cords have been markedly increased in diameter. The cells constituting these cords, however, have been converted into a cornified type of cell totally different from normal secreting prostate cells. Peripheral cells in the cord have been somewhat less markedly changed than those nearer to the lumen. The collapsed character of the u-g sinus walls (urethra) is markedly different from those receiving androgenic treatment.

It becomes evident that the prostate epithelium of this stage of differentiation can be rapidly stimulated to differentiate into typical adult secretory cells by androgens, or undergo materially different development from estrogenic treatment; it is also evident that at this age testes can be awakened to rapid hormone secretion by the gonadotropic substance of pregnancy serum.

IV. DISCUSSION

When considering the phase of this study relating to testis removal we are provided with the first successful embryonic castration in a mammal. Whereas technically the pouch young individual is no longer an embryo, the state of development of its reproductive system on pouch day 22 is equivalent to that occurring during intrauterine life in other mammals; prostate development corresponds quite closely with that in the rat on the nineteenth day of gestation.

The results described show clearly that prostate development is but mildly slowed, if at all, by removal of the testes. While estimates arrived at by very careful analyses indicate a smaller amount of total prostate tissue in the operated than in the untreated control, it is unknown how much of this is due to the rigors of the operation, or to individual animal variability, or whether any of the partial retardation is to be attributed to loss of substances normally provided by the testes. The fact that development has continued along normal lines, that the prostate tissue has increased by 3500% within the 28 days since castration, and that despite a severe operation a prostate 66% to 71% the size of an untreated brother from the same pouch has developed, clearly reveals that prostate development and growth in the opossum does not depend upon secretions produced in the testes during these early developmental stages.

These findings on prostate development call to mind the demonstration by Price ('36) that castration of the rat at birth does not prevent further development of the prostate, nor prevent it from attaining the secretory differentiated state which is indicated by the development of light areas in secreting acinus cells; such a state of development was attained only slightly later than in normal littermate controls. Price ('39) has summarized the existing evidence to suggest that an influence, androgenic in nature, emanates from the suprarenal gland of the rat that is effective until about day 35 after which it is no longer exercised and prostate secretory involution sets in. Suggestions for this hypothesis are likewise embodied in the publications of Howard ('37, '38), Davidson and Moon ('36) and Burril and Greene ('39).

In work with opossum development and the findings as to effects of sex hormone on the process of sexual differentiation (Moore, '41) general considerations led to the formulation of a working hypothesis that sex ducts and accessory organ differentiation, at least during the definitive stages, proceeded without regard to the production of gonad hormones to direct such differentiation; and that instead, the genetic

factors, possibly involving circulating and stimulating substances provided by the organism as a whole, were the guiding forces in differentiation. Up to the present time no evidence from the opossum points to the localized formation of stimulating substances, either in the testes or in the suprarenals. And until suggestive evidence for such localization is in hand it is preferred to think in terms of organismic influences as a whole. Substantial proof of the production of secretions by the early developing gonad, that act as guiding influences on sexual differentiation, is yet to be found.

The second phase of the present report adds several points to hormonal responses in the developing reproductive system that were presented earlier (Moore '41). First to be mentioned is that the prostate gland during its later embryology is capable of rapid response to androgenic stimulation and assumption of adult secretory conditions, at an age of 5 months; this is brought into evidence clearly by t-propionate treatments, and is as clearly indicated by treatments with gonadotropic hormones. This introduces the second point, namely, that by the age of 5 months testes have become sensitive to such gonadotropic agents. It had been reported earlier, on the basis of treatments at different ages, that all applications of gonadotropic hormones to males or females up to approximately day 100 were largely ineffective. More experiments are required to establish with certainty the state of maturity of the gonads required for response to gonadotropic substances. Present results clearly demonstrate that by the fifth month gonads can respond with precocious secretion of sex hormones. A third point relates to the character of response of the urogenital sinus or prostate outgrowths when subjected to the influences of estrogenic hormones. It was previously shown that in stages of differentiation existing between days 30 and 100 hyperplasia and metaplasia of urogenital sinus epithelium, and prostate cords if formed, invariably followed estrogenic treatment. Present findings show little change in the character of response of these tissues at

the more advanced developmental stage in the 5-month young. The entire prostate cords are markedly changed from the stage of their differentiation attained up to this period by daily estrogenic treatments of less than 2 weeks duration. A fourth, and final, point reveals the fundamental change in potencies of the urogenital sinus epithelium of females. It was demonstrated earlier that androgenic treatments of young females induced the appearance of typical prostate outgrowths, which never appear in normal female development. Androgen treated females produced prostates more advanced in development than those in untreated normal males of the same age. Furthermore, up to a period of 95 days in the pouch, prostates of well developed types were present in treated females. In contrast, 5-month-old females receiving daily treatments of 2.0 mg. t-propionate showed no evidence of prostate bud formation after 12 days of treatment. Whether the capacity to produce prostate tissue on the part of the female urogenital sinus epithelium has been totally lost, and if so, at what stage of differentiation this occurred, must be left to subsequent experiments to reveal.

V. SUMMARY AND CONCLUSIONS

1. Successful testis removal from pouch young opossums on day 22 (equivalent to embryonic castration in placental mammals) is followed by typical prostate gland differentiation. Castration on day 22, with sacrifice on day 50, reveals an increase of prostate tissue in the castrate of 3500% and in the normal of 5000%, or a prostate increase in the 28-day castrated male of 66% to 71% of the amount of increase in the normal male.

2. Prostate differentiation and growth are not dependent on gonadal-secreted hormones up to day 50.

3. Prostate differentiation in 5-month-old males is quickly modified to the adult secretory condition by t-propionate. Estradiol induces great hyperplasia and metaplasia of prostate tissue.

4. Testes of 5-month-old young respond to gonadotropic stimulation, and precociously secrete male hormone. This induces typical precocious prostate development.

5. The urogenital sinus of females, responding at early pouch young stages to androgens by typical prostate induction and precocious development, failed to differentiate prostate tissue after twelve daily treatments with 2.0 mg. t-propionate at an age of 5 months.

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PLATE 1

EXPLANATION OF FIGURES

2 Photomicrographs of cross section through prostate area of urogenital sinus of opossum pouch young. Originals $\times 100$. A, Prostate primordium in normal 24-day male (op.ser. 10B). B, Prostate cords of 50-day male, castrated on day 22 (op. ser. 159). C, Prostate cords of normal 50-day male (op. ser. 160).

3 Photomicrographs of cross sections of prostate area of urogenital sinus of 5-month-old opossums (through upper one-third of each shown in fig. 2). Originals $\times 20$. A, Normal. B, Gonadin treated. C, T-propionate treated. D, Progynon-B treated.

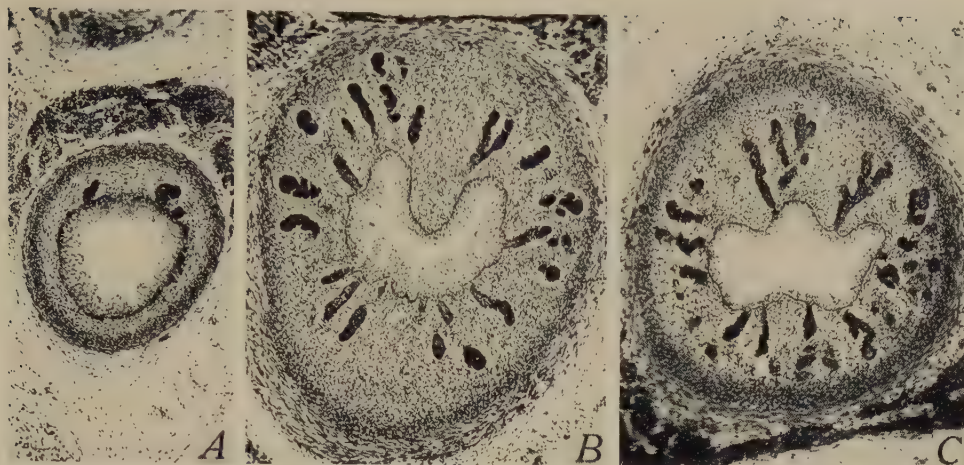


Figure 2

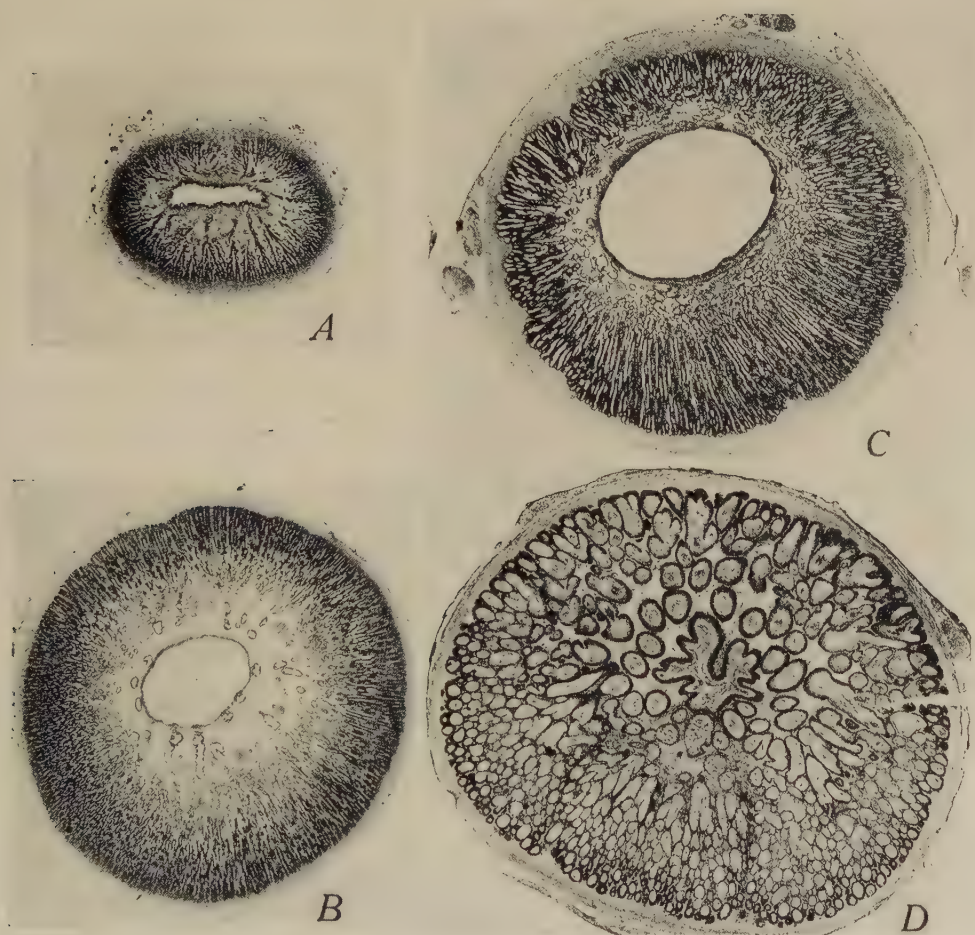


Figure 3

THE APPLICATION OF THE ALTMANN METHOD TO THE STUDY OF THE GOLGI APPARATUS ¹

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TWO PLATES (EIGHT FIGURES)

Forty odd years ago Golgi (1898) described the intracellular reticular apparatus that has since borne his name. In the intervening years, probably no other cellular structure has called forth such a barrage of papers—literally many hundreds in attempts to explain its nature and function, and to debate its actual existence. This mass of literature has been adequately summarized in the reviews of Duesberg ('14), Cajal ('14), Bowen ('26), Kirkman and Severinghaus ('38), and others. These should be referred to for a general survey of the problem.

In seeking for points on which a majority of investigators agree, one finds that upon no single fact concerning the nature and function of the Golgi apparatus can unanimity of opinion be said to exist. Possibly the closest approach to a generally acceptable idea is that the Golgi apparatus is a true morphological component of most living cells. Even this idea, however, has an opponent in the school of Ciaccio ('29) which holds that the Golgi apparatus, canals of Holmgren, neutral-red bodies of Parat, etc., are all mere functional states or technical artefacts produced in a specialized region of the cell, the "zone of Golgi."

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Recent advances in cytological technique have made it possible to study the Golgi apparatus by newer methods. The result of a study by the modified Altmann method is presented in this paper.

MATERIAL AND METHODS

Since much of the confusion appears to have been due to difficulties in interpretation of findings in invertebrate material and of findings in vertebrate sex cells during the process of spermatogenesis and oögenesis, it seemed desirable to confine the introductory studies in the realm of this new technic to tissues in which the Golgi apparatus had been intensively studied, and in which the morphological relations with other cytoplasmic constituents were more or less generally agreed upon. The tissues chosen were the epididymis, spinal ganglion, pancreas, duodenum, and kidney of the guinea pig, and the spinal ganglion of the rabbit.

Tissues were prepared according to the methods of freezing-drying, freezing-thawing, and freezing-substitution following the procedure and observing the precautions outlined in another paper (Simpson, '41).

Tissues studied were from normal animals, from animals dehydrated by withdrawal of food and water, and from animals which had been injected with varying quantities of neutral red preceding killing.

The tissues were examined in free hand sections and in sections imbedded in paraffin or celloidin. The free hand sections were examined in a dark room with the eyes shielded from extraneous light. This was found necessary in order to observe the delicate structural differences in the cleared, unstained tissues. Studies on solubility were carried out immediately after dehydration to avoid the gradual denaturation of protein known to occur as the dry blocks stand in a desiccator (Bensley and Hoerr, '34).

Throughout the study control tissues were prepared by the usual cytological methods and by methods for the demonstration of the Golgi apparatus. For the former, Zenker-

formol, Regaud's fluid, Bouin's fluid, 5% trichlorolactic acid, 5% trichloroacetic acid, and other fixatives were used. The methods used for the Golgi apparatus were the Kolatchev-Nassonov technic, the Mann-Kopsch method, and the mixture of 0.5% ferric chloride in 2.0% osmic acid which was found to give impregnations of comparatively high constancy by Owens and Bensley ('29).

OBSERVATIONS

Guinea pig epididymis. Free hand sections of the epididymis examined after clearing with mineral oil in vacuo appeared to be very well preserved (see fig. 6). This was particularly true of cells from tubules near the surface of the block of tissue. In the cells of what Benoit ('26) called "the portion of the tube following the initial segment", it was observed that the granularity of the cytoplasmic background depended upon the quality of the freezing. In the preparations reported here, this granularity was usually very delicate. The oval nucleus, lying toward the basal pole of the cell, was frequently seen to contain several chromatin particles of various sizes. Mitochondria were readily visible, usually as fine filaments lying parallel to the long axis of the cell. At the distal end of the nucleus secretion droplets tended to be accumulated, an occasional one of which appeared highly refractile.

Dark room observation revealed in the proximal part of the region between nucleus and free margin of these cells a zone from which mitochondria were for the most part absent. This zone appeared to be in the form of a slightly less dense ovoid area. Careful observation with light of reduced intensity showed that this area was not homogeneous but consisted of a coiling tubule or tubules of rather narrow diameter. No membrane was seen bounding this light canalicular structure.

Examination of this region of the cytoplasm with the aid of reflected light of the Ultropak microscope, with the cardioid dark field condenser, and with the polarizing microscope did not yield further useful information. Sections at tempera-

tures well below the freezing point of intracellular lipoids showed no new points of luminescence when examined with crossed Nicoll prisms.

In cells from animals which had received neutral red injections, the granules of red-stained material appeared first and most numerous at the distal end of the nucleus, in the region where secretion granules were also seen (fig. 6). These granules were not connected with the intracellular canals described above. They might, when more numerous, be found in any part of the cell, the greatest accumulation remaining always just distal to the nucleus and running over the canals described above. The use of polarized light revealed that these granules were not for the most part anisotropic. Occasionally, however, anisotropic granules were seen.

These same canals appeared uncolored in free hand sections stained in Hansen's chrome-alum-hematoxylin and eosin. In such preparations the "negative image" corresponded exactly with the "negative Golgi" seen in well fixed cytological preparations stained with deep cytoplasmic dyes. The appearance of the structure was not altered in free hand sections treated for a few minutes before staining with alcohol, acetone, or normal saline.

This "negative image" appeared in frozen-dried materials denatured in absolute alcohol or by heat and stained with hematoxylin and eosin, toluidin blue, or other cytoplasmic dyes. In embedded materials where thinner, more uniform sections could be cut, the canal, so far as it was possible to determine, was continuous and without anastomoses between adjacent loops (fig. 1). Sections cutting across the cell through this region showed the canals to be uniform in structure. The shape in this plane was an almost closed circle, the ends of which appeared not quite to meet but rather to run up or down into the cell.

Studies upon frozen-dried epididymis from which all extractable lipoids had been removed by boiling in absolute alcohol for 24 hours and subsequent boiling in anhydrous chloroform for a like period showed that this procedure caused

no serious morphological change within the cytoplasm. The general appearance of these sections was very similar to that of material which had not been subjected to such vigorous extraction. When such sections were stained with a freshly made solution of Weigert's resorcin-fuchsin stain for 24 to 48 hours at 37°C., there appeared with low power examination a darkly stained structure in the region previously observed to contain canals. With high powers of the microscope this structure could be seen as an anastomosing group of delicate threads in cells of the tubules near the surface of the block (fig. 5). These threads frequently appeared to lie within ill-defined canals in the cytoplasm. The refractoriness which the tissues prepared in this fashion showed toward other dyes as counterstains for the cytoplasm made it difficult to determine the extent of these canals and their relation to the negative Golgi. The threads did not stain as distinctly in tubules slightly removed from the surface (fig. 4). Within the innermost tubules of even a rather small block no such threads could be seen. In this region of the cytoplasm of these cells there was no evidence of the existence of either canals or threads since this zone was broken by ice crystal formation to a greater degree than the rest of the cytoplasm.

Attempts at impregnation of frozen-dried blocks in osmic acid or its vapor proved entirely unsuccessful. The reduction of osmic acid in these preparations was in the form of a finely dispersed blackening of the entire cell.

Preparations made by freezing and then thawing in the fixatives used for Golgi apparatus, followed by osmic acid impregnation showed in general a tendency of the reduced osmium to be diffusely distributed in the cells, the background seldom being as clear in these preparations as in cells from unfrozen tissues. On the margin of the sections of this material could be found quite satisfactory impregnations of the Golgi apparatus. They were never as sharply circumscribed as the best conventional preparations, and tended to fade off somewhat hazily into the general cytoplasm. As tubules slightly removed from the surface were examined,

it was noted that in the region of the apparatus there was a blur of osmium but that the characteristic form was lost. Here the Golgi apparatus appeared to be broken into large granular pieces. The inside of the tissue, considerably removed from the surface of the block, presented a rather granular cytoplasm but otherwise no clear evidence that the tissue had been frozen before fixation. Particularly in preparations made by following the Kolatchev-Nassonov technic after freezing, there was a uniform tendency for the outlines of the Golgi apparatus to be sharply delimited with osmium while in practically no cells was the inside of the apparatus blackened.

Rabbit and guinea pig spinal ganglia. Free hand sections of spinal ganglia revealed a response to freezing that was unique. The bits of tissue were usually so small that the factor of relatively slow freezing was minimized. There was seen, nevertheless, a wide range in size of ice crystal artefacts in the different cells of each ganglion. The distribution of cells showing large artefacts and of those with but a finely reticulate cytoplasm appeared to be purely random. The only observed fact associated with this was that the large cells of the ganglion usually showed much coarser reticulation than did the small cells.

Within the smaller, better preserved cells, the karyoplasm might be found to be homogenous except for delicate reticulation. Other nuclei showed a variable degree of reticulation due to ice. When unstained, the cytoplasm appeared finely granular, with its contained mitochondria in the form of short filaments and granules. In the best preserved cells, observed under conditions allowing the eyes the maximum of sensitivity, the zone between the nucleus and the border of the cell was seen to contain a twisted, apparently continuous intra-cytoplasmic canal less opaque than the rest of the cytoplasm. The extent of this perinuclear canal varied from cell to cell. In general, however, it may be said that the structure did not quite touch either the nucleus or the outer wall of the cell.

Nissl bodies were not visible in the unstained cell. When aqueous solutions of toluidin blue were applied to undenatured free hand sections, the ice crystal artefacts tended to close up and produce a smooth cytoplasm as Hoerr ('36) reported. In the cytoplasm Nissl bodies were seen to fill almost completely the perinuclear area except for the space occupied by the coiled Golgi apparatus. Both the Nissl bodies and the canals between them were resistant to brief treatment of the free hand section by alcohol, ether, acetone, or normal saline before staining. Observations on denatured spinal ganglia paralleled those on free hand sections of freshly dried tissues except for the fact, that ice crystal artefacts were rendered permanent by the denaturation (fig. 8).

When alcohol denatured material was stained with resorcin-fuchsin, a network of fine anastomosing fibers was visible in the region formerly seen to contain canals (fig. 7). After treatment of the section with this dye, Nissl bodies were refractory to staining and their exact relation to the net has not as yet been determined. In some places, however, canals or their remnants appeared to be left in the cytoplasm. Where it was possible to make these out, it was usually found that the fine darkly stained threads lay along the inside surface of the canal (fig. 7 a); in some instances threads were seen along both inside walls (in profile). In the most clean-cut cases, the picture presented by the resorcin-fuchsin stained cells of the spinal ganglia (fig. 7 b) might be almost exactly superimposed upon the plates used by Golgi (1898) to illustrate his internal reticular apparatus in spinal ganglion cells of the cat. As the freezing reticulum became coarser in larger cells, the resorcin-fuchsin stained threads became less distinct and tended to lose their identity among the strands of the reticulum. Nissl stains on the less well preserved cells, on the other hand, continued to demonstrate canal-like interstices between Nissl bodies.

Guinea pig pancreas. Most of the studies reported have been carried out on the spinal ganglion and epididymis. In a few instances, however, in cleared free hand sections of

pancreas a delicate clear net among the zymogen granules of acinar cells could be observed. This net exhibited a morphology that has come to be accepted as the normal resting condition of Golgi apparatus in these cells.

In a few cases thin free hand sections were obtained in which islet cells could be observed. Here a canalicular system comparable to that observed and pictured in the living cell by Bensley ('11), O'Leary ('30), and Beams ('30 b) could be made out. The delicate granules of the islet cells facilitated the outlining of the apparatus.

Guinea pig duodenum. In well frozen specimens of duodenum, the ice crystal artefacts were so small that they were invisible with the highest powers of the microscope. The epithelial cells were a favorable site for study of cytoplasmic organoids. In cleared free hand sections of this tissue there could be seen between the nucleus and the striated border a delicate canalicular system, clearer than the rest of the cytoplasm. This corresponded in position and morphology to the "negative Golgi" of fixed material and to the cleanest positive impregnations of the apparatus in these cells.

DISCUSSION

Throughout this report the term Golgi apparatus has been used on the strength of its general acceptance, and not because the present state of knowledge permits reading into the term "apparatus" any very definite concept of physical structure.

It is probably true, as Kirkman and Severinghaus ('38) point out, that today the majority of cytologists reject any canalicular or vacuolar interpretation of the Golgi apparatus. The most generally accepted view appears to be that held by Golgi himself, and supported at great length by Nassonov, Bowen, Gatenby, and Ludford, that the apparatus is a highly specific compact substance of thickly fluid or semisolid nature. Traditionally there usually accompanies this theory an inference, based on not too satisfactory evidence, that this substance is lipoidal in nature. The only alternate view that

has received any measure of support over a considerable period of years, is that the structure is a canalicular system homologous with the vacuole of plant cells. This opinion was expressed first by Bensley ('10), and has since been supported mainly by the school working with him and by plant cytologists following the lead of Guillermond ('27).

With regard to the neutral red bodies, it can merely be stated that in the present investigation it was possible to give further confirmation to the findings of Beams ('30 a, '30 b) and Chang ('35) that there is no more topographical relation between the granules formed by neutral red and the canalicular apparatus in the cells of epididymis than between neutral red granules and mitochondria, nucleus, or other cell organoids (fig. 6). Chang's important observation that the neutral red bodies blacken in osmic acid in a few hours instead of the days taken by Golgi apparatus was also confirmed.

Most cytologists are now inclined to agree that the Golgi apparatus is not a fixation artefact, even though their concepts of its nature may vary widely. Occasionally a note of doubt on this point is expressed in the literature (Walker and Allen, '27, and subsequent papers noted by Kirkman and Severinghaus, '38). The few observations of the Golgi apparatus in living cells have, because of their mutual agreement, been generally accepted as unquestionable evidence of its reality prior to fixation. To these may now be added, from study of cells whose activities have been almost instantaneously stopped by physical means, four more sites where a canalicular apparatus has been observed in forms similar to the negative images seen in well fixed material and to the pictures seen in the cleanest osmium impregnations. The report of its presence in another cell is also confirmed, namely the islet cell of the pancreas of the guinea pig.

Examination of the cases in which the Golgi apparatus has been reported shows that its morphology fails to give any hint of a solid, or semisolid structure. Concepts of the Golgi material as thickly liquid are a compromise found necessary

to explain the failure of microdissection studies to reveal any area in the cytoplasm which offered resistance to the passage of the needle (Cowdry, '24, quoting Kite and Chambers). The observation of a uniformly clear set of canals in cases where Golgi apparatus has been identified in living cells and in frozen-dried cells correlates well with the general failure to stain the Golgi apparatus in well fixed cytological material by any other means than the highly dubious technic of impregnation by colloiddally dispersed metals (Owens and Bensley, '29).

It is my opinion that the different forms reported for the Golgi apparatus are more often due to artefacts of technic than to physiological variations. In view of this, and of the confirmation by fresh tissue studies of the reality of the smoothly outlined cytoplasmic canals observed in both frozen-dried and in other well fixed materials, it is suggested that the evidence of the current findings favors the canalicular concept of Golgi apparatus. Obviously, on the strength of investigations confined to animal tissues, one can make no statements regarding the original homology with the plant vacuole as proposed by Bensley ('10).

The observations here reported on the stainability of the Golgi apparatus after extraction from the cell of alcohol and chloroform soluble lipoids indicate that any concept portraying the apparatus as composed entirely of lipoids is inaccurate. The enhanced stainability of what we believe to be part of the apparatus after vigorous lipoid extraction suggests the presence of a protein component in such a diffuse state that it may be revealed by staining only after aggregation contingent upon removal of lipoids. This fact also suggests that the apparatus contains a highly diffuse lipoid, so highly diffuse in fact that it may not be revealed by ordinary methods for demonstration of lipoids. The generally high susceptibility of the region of the Golgi apparatus to "blowing up" with freezing suggests that the fluidity of the apparatus may be high.

The observations on the osmium impregnation of frozen-thawed tissues also supports this contention. In the cells at the surface of the block, where freezing was found to be best, an intense positive impregnation followed use of the technic. In the adjacent zone, where ice crystal artefacts of freezing were most apparent, the disruption of the Golgi apparatus persisted in thawing even more than did the disruption of the general cytoplasm. The inner zone again was capable of the usual impregnation, although the canals failed to become filled with osmium. The higher susceptibility to disruption in freezing of the Golgi region in the second zone tended to indicate that the Golgi apparatus was more fluid than the more condensed adjacent cytoplasm.

CONCLUSIONS

1. The Golgi apparatus is a morphological entity within the cells of the guinea pig epididymis, pancreas, duodenum, and the rabbit spinal ganglion. Its appearance is that of a slightly less dense intra-cytoplasmic canal in all the tissues studied.
2. Evidence is presented in support of the concept that the Golgi apparatus is a system of fluid filled canals of relatively uniform bore. The material studied showed no evidence as to the nature of the cytoplasmic interface that exists between Golgi apparatus and enveloping cytoplasm.
3. The uniform failure of attempts to impregnate the Golgi apparatus of frozen-dried tissues by osmic acid indicated either that no specific "osmiophilic" Golgi "substance" exists or that if it does, its properties are markedly altered by freezing-drying.
4. The Golgi apparatus retains a stainable component after prolonged extraction in boiling lipid solvents. This we assume to be protein in nature. The enhanced stainability of this constituent after alcohol and chloroform extraction indicates the presence of some dispersive lipid in the Golgi apparatus.

5. Confirmation is offered of the de novo origin of neutral red bodies. No significant morphological relationship exists between the neutral red granules and the canalicular apparatus in the cells studied.

I am indebted to Miss Agnes Nixon of the Department of Anatomy of The University of Chicago for the illustrations.

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PLATE 1

EXPLANATION OF FIGURES

Figures 1-6 Guinea pig epididymis. All drawings were made originally under oil immersion at a magnification of $\times 1440$.

1 Altmann technic. Alcohol denatured. Celloidin embedded. Stained with hematoxylin-eosin-azure. Note the clear canals distal to the nuclei.

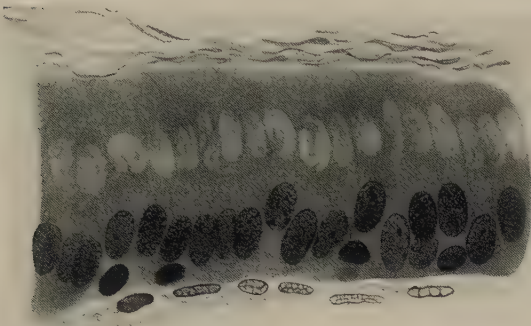
2 Regaud fixed, stained with aniline-acid-fuchsin and methyl green.

3 Normal Golgi apparatus as seen in a Kolatchev-Nassonov preparation.

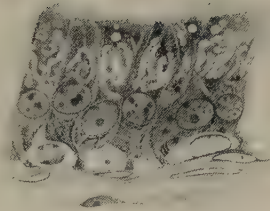
4 Altmann technic. Dried block extracted with hot alcohol and chloroform as described in text. Stained with resorcin-fuchsin. These cells are from a tubule somewhat removed from the surface of the block. Note the coarse granularity of the region of the Golgi canals and the indistinct strands stained by resorcin-fuchsin in this region.

5 Prepared as figure 4. Cells from a tubule near the surface. Only a part of the stained strands have been drawn in order to show more clearly their relation to the canals of the Golgi apparatus.

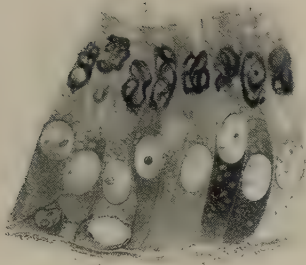
6 Free hand section of epididymis prepared by Altmann technic. Guinea pig had been injected with neutral red prior to killing. Note the position of the neural red granules in relation to cellular constituents and the relative freedom of the region of the Golgi apparatus from such granules.



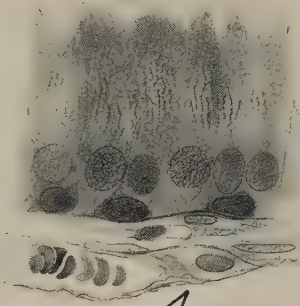
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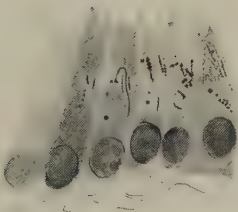
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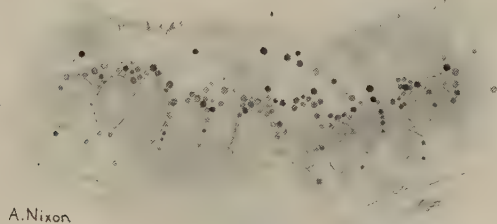
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5



A. Nixon

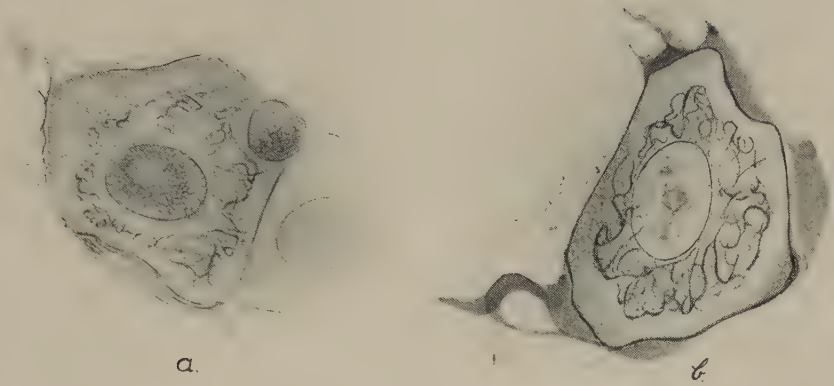
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PLATE 2

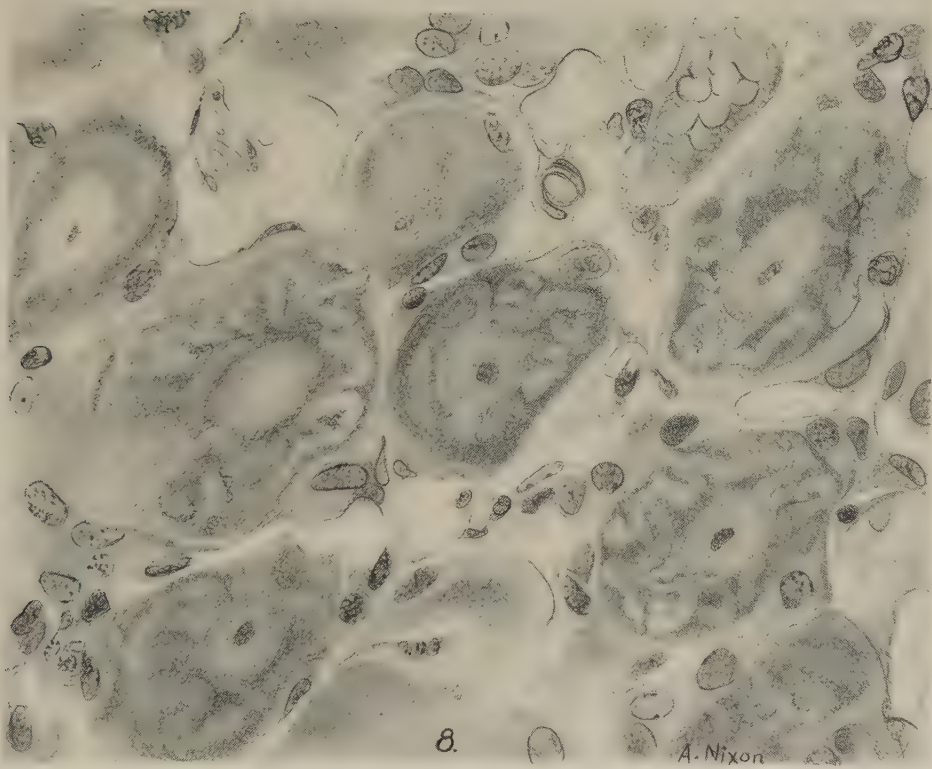
EXPLANATION OF FIGURES

7 Guinea pig spinal ganglion cells. Altmann technic. Extracted with alcohol and chloroform. Resorcin-fuchsin stained. The delicate network of stained fibers resembles very closely the picture frequently obtained by silver or osmium impregnation of the Golgi apparatus. 7 a shows the relation of some of these strands to clear cytoplasmic canals.

8 Same tissue. Celloidin section stained with toluidin blue and erythrosin. Clear canals within the perinuclear cytoplasm are very clearly evident in such preparations and occupy the same relative position as do osmicated nets in the best impregnations of the Golgi apparatus.



7



A. Nixon

THE DIFFERENCE IN THE RESPONSE OF SKELETAL TISSUES TO ESTROGEN IN MICE OF VARIOUS AGES ¹

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TWO PLATES (SIX FIGURES)

In previous investigations ('39, '40) we have shown that the response of cartilage and bone of guinea pigs to stimulation by hormones is, among other factors, determined by the age of the animal at the beginning of the treatment. The first reaction of the epiphyseal cartilage to estrogen consisted in a suppression of its growth. This suppression was more marked in newborn guinea pigs treated with estrogen, than in older immature guinea pigs injected with estrogen. As we have found since ('41 b), in growing mice treated with estrogen the early effect of this hormone on the skeletal tissues consists likewise in an inhibition of proliferation of the epiphyseal cartilage, and in addition in an inhibition of absorption and in an increase in apposition of bone around spicules and shaft. At later stages the appositional processes ceased if estrogen was continuously administered, while resorptive processes became manifest and subsequently increased. This change, which took place in mice under prolonged influence of estrogen, corresponded to the increase in absorptive processes seen with advancing age in normal mice (Silberberg and Silberberg, '41 a). The present investigation was made to deter-

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mine whether the effect of estrogen is modified if the hormone is administered to old, instead of to young mice.

MATERIAL AND METHODS

Most of the material was secured from experiments which had been carried out in this laboratory for other purposes. We are greatly indebted to Dr. Leo Loeb for his interest and advice in this investigation.

Tibiae and femurs of seventy-two mice, nineteen males and fifty-three females, belonging to the closely inbred strains C₅₇, CBA, New Buffalo, Old Buffalo, D, and C₃H were studied. The animals were divided into two groups according to their age; one group consisted of fully grown mice, 6 to 11 months old, and the second group of older mice, 12 to 21 months old at the beginning of the experiment. In both groups a number of animals received a weekly dose of 100 RU of estradiol benzoate in oil (Progynon-B Schering) subcutaneously, and others, a weekly dose of 200 RU; the latter was, in some cases, reduced to 150 RU if the weight of the animals was less than or had dropped to below 20 gm. The duration of the experiments varied from 2 week to 8 months. Details concerning the distribution of strains, sexes and experimental periods are given in tables 1 and 2.

At autopsy, tibia and femur were removed as a whole, and sections were prepared for histological study.

For the purpose of comparing the effects of estrogen in the various age groups, the bones of growing mice obtained in former experiments served as additional control material.

MICROSCOPIC EXAMINATION

The following description in all three age groups is based upon the findings in mice of strains C₅₇, CBA, New Buffalo and Old Buffalo, which, under normal conditions, show approximately the same slow rate of skeletal ageing.

I. Young mice, 2 to 13 weeks old at the beginning of the experiment

In a previous paper ('41 b), we reported on the changes taking place in the long bones and joints of growing mice

subsequent to the administration of estrogen. The main changes, seen in those experiments, may be summarized as follows:

TABLE 1

Adult animals, 6 to 11 months old at the beginning of the experiment.

	NO. OF MICE	100 RU ESTROGEN PER WEEK						150/200 RU ESTROGEN PER WEEK					
		C ₅₇		Old B.		D		C ₅₇		C ₃ H		D	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
½ month	5				2			3					
1 month	6		1					4					1
2 months	8							2		3		1	2
3 months	7				2			2		1			2
4 months	5	1	1			1				2			
5 months	5		1							1		3	
6 months	1	1											
8 months	2	2											
Total	39	4	3		4		1	11		7		4	5

TABLE 2

Older animals, 12 to 21 months old at the beginning of the experiment.

	NO. OF MICE	100 RU ESTROGEN PER WEEK				200 RU ESTROGEN PER WEEK							
		C ₅₇		CBA		C ₅₇		CBA		New B.		Old B.	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
½ month	5					1		2		1		1	
1 month	2							2					
1½ months	4					1				2		1	
2 months	5							1		2		2	
3 months	2							2					
4 months	7		1		2	2		2					
5 months	5		1		2			2					
6 months	1							1					
7 months	1							1					
8 months	1							1					
Total	33		2		4	4		4	10		5		4

(a) *Epiphyseal disk.* The cartilage underwent increased hyalinization, sclerosis, calcification and destruction. The proliferation of the resting cartilage cells and their differentiation into columnar cartilage cells, and of the latter into hypertrophic cartilage cells were inhibited, as was the destruction of the hypertrophic cartilage cells by capillaries; in con-

trast to the normal mechanism of replacement of the destroyed cartilage by bone, a metaplastic conversion of sclerosed cartilage into bone occurred. On account of the increased sclerosis and of the inhibition of the absorption of cartilage, the onset and progress of epiphyseo-diaphyseal union were delayed. Under a weekly dose of 200 RU of estrogen, the maximum changes were seen after administration of 2600 RU of estrogen. Subsequently, resorptive processes were resumed and led to perforations of the epiphyseal disk. However, even after 9 months of treatment with estrogen, the degree of epiphyseo-diaphyseal union reached was not advanced over that observed under normal conditions.

(b) *Metaphysis and diaphysis.* During the earlier periods the matrix of the bone marrow, particularly around the spicules, had become denser and had increased in amount; absorptive processes leading normally to a removal of the trabeculae were decreased; an excessive amount of bony tissue was present in the subchondral zone, forming an osseous network, in the meshes of which congested capillaries and an accumulation of epithelioid cells and megakaryocytes were seen. Under continued treatment with estrogen, this bony tissue encroached upon the inner surface of the diaphyseal shaft as well as upon the marrow cavity. When this process had reached its maximum, a dense interlaced bony network partly occluded the marrow cavity. With the resumption of resorptive processes the excess bone was dissolved, and conditions gradually returned to normal or almost normal. In the lateral protuberances of the growth zones and in the bony shaft itself, the changes took a similar course. The amount of bony tissue was at first increased, and later, with the predominance of the resorptive processes, a thinning became noticeable. The periosteal tissue was, in the early stages, thickened and more fibrillar; subsequently it became loosened and participated in the absorption of the cortex.

(c) *Joint.* The articular cartilage cells underwent a temporary hyperplasia and hypertrophy; later, the intercellular substance became markedly sclerosed and hyalinized and the

incidence and severity of arthropathic changes seen in normal old mice was greatly reduced.

II. *Adult mice*

Depending on the age of the animal at the beginning of the treatment and on the duration of the latter, the age of these animals at autopsy varied from 6½ to 19 months.

In *untreated mice*, during this period, the epiphyseal plate is represented by a continually narrowing layer of non-proliferating, increasingly hyalinized, calcified or sclerosed cartilage, in which the cells have lost the columnar arrangement and structural characteristics of differentiation as seen in growing animals. Amorphous material and osseous plugs have replaced degenerated cartilage cells. The epiphyseal cartilage is separated from the bone marrow by a transverse osseous lamella, which at first increases and later decreases in thickness and density. During the second half of this period, resorptive processes originating from the bone marrow make progress and perforations of the transverse bony plate and of the epiphyseal disk may be noted, but the degree of epiphyseodiaphyseal union remains limited. The metaphyseal spicules are gradually dissolved. The bony shaft increases in thickness during the first year of life; after that period, the compacta becomes thinner but remains solid.

Effects of estrogen in adult mice, 6 to 11 months old at the beginning of the experiment

(a) *Epiphyseal disk*. After administration of a total dose of 200 to 800 RU of estrogen, given over a period of from 2 to 4 weeks, calcification, sclerosis, destruction and ossification of the epiphyseal cartilage were increased over the normal, but the increase was less than in growing mice under corresponding treatment. Whereas in growing mice the resorption of the cartilage by bone marrow was markedly inhibited under the influence of estrogen, in the adult animal the resorptive processes were not decreased, as compared with the normal.

These changes were more pronounced after injections of a weekly dose of 200 RU than of 100 RU of estrogen.

A total amount of about 1500 RU of estrogen, administered over a period of 3 or 4 months, which in growing animals led to the appearance of a great amount of bone, did not cause any appreciable progress in the ossification of the cartilage in the adult animals; instead, resorptive processes had caused perforations of the epiphyseal disk, which were much wider and more numerous than in growing mice similarly treated, or in untreated adult animals at this age. Thus, in a 13½ months old C₅₇ mouse which had received 1700 RU of estrogen over a period of 4 months, the structural age of the epiphyseal disk and the degree of epiphyseo-diaphyseal union were comparable to those seen in untreated mice about 2 years of age.

With the duration of the experiment increasing up to 8 months, and the estrogen administered amounting to about 4000 RU, the ossification of the cartilage made no progress over that seen at the earlier stages, but processes of resorption were active. However, the structural age of the epiphyseal disk and the degree of epiphyseo-diaphyseal union in a 19 months old male C₅₇ mouse, which had received 3500 RU of estrogen over a period of 8 months, were only slightly increased over those of a 13½ months old mouse treated with estrogen for 4 months, and this animal resembled already more closely its normal untreated control than the former mouse did.

Correspondingly, even in those instances in which the epiphyseo-diaphyseal union was farthest advanced under the influence of estrogen, it had not progressed beyond the maximum observable in normal old mice.

It may be mentioned, and this holds good for both groups of animals—the young as well as the adult mice—that the degree of the changes seen under the influence of estrogen was not always the same in the tibia and femur. In some instances the tibia was more affected, in others the femur.

(b) *Metaphysis and diaphysis.* A dose of 100 RU of estrogen, injected weekly for 2 weeks, had no effect, and if injected

for 4 weeks, only a slight effect on the subepiphyseal tissue was noted, whereas, in young mice under similar conditions considerable amounts of bone had already been formed. Administration of 200 RU of estrogen weekly for 2 to 4 weeks (fig. 1) caused a definite thickening of the bone underneath the epiphyseal cartilage, which, however, was less accentuated than in growing mice treated correspondingly. The bone marrow contained enlarged and congested capillaries, the contents of which were clotted or hyalinized, and the more so the closer the vessels were situated to the epiphysis. The reticulum cells proliferated vigorously by way of mitosis. Particularly along the bony spicules and around the capillaries the connective tissue gradually increased in amount and density, and it contained epithelioid cells in greater numbers than usual. Blood-forming cells and some megakaryocytes likewise proliferated by way of mitosis. Other megakaryocytes were greatly enlarged, stuck together, and coalesced. Simultaneously, they suffered retrogressive changes consisting at first in homogenization of the cytoplasm, later in pyknosis, karyorrhexis and karyolysis.

Whereas, in normal mice 12 months of age all or almost all of the spicules are dissolved and the diaphyseal marrow cavity is filled with hemopoietic marrow, under the influence of estrogen the picture was different: many trabeculae were preserved. The epithelioid cells in the metaphysis were numerous, some of them, acting as osteoblasts, arranged themselves at the periphery of the spicules in a bead-like manner and were converted into osteocytes. Thus, an interlaced bony network was formed, connecting the trabeculae. It differed from that seen in younger mice injected with estrogen in that it was looser, probably owing to the smaller number of spicules present in the older mice when that treatment was begun. As in growing mice, but to a lesser extent, this bony tissue encroached upon the inner surface of the bony shaft and upon the marrow cavity, partly occluding the latter. However, in contrast to conditions in growing mice, resorptive processes were already seen at this stage in the adult group; epithelioid

cells of the bone marrow coalesced, forming osteoclastic giant cells, which, in cooperation with capillaries and proliferating connective tissue, dissolved the excess bone. A richly vascularized, in part loosely fibrous, in part dense osteoid, tissue had replaced the ordinarily hemopoietic marrow. Numerous blood extravasates and deposits of blood pigment were absorbed by phagocytes. Thus the marrow cavity had assumed a variegated appearance.

If the administration of estrogen was continued still longer, the resorptive processes began to predominate more and more, and in some mice, 18 to 19 months old and injected with about 3500 RU of estrogen over a period of 8 months, resorption had progressed to such an extent that, as far as the bony constituents are concerned, the histological picture of the marrow cavity could be almost normal; in others, however, there were excessive amounts of bone still present, but they were in advanced stages of absorption.

The increased bone formation in the diaphysis coincided with an increased density of the periosteal tissue. Osteoblastic epithelioid cells were found in close approximation to the bone, but they were not more numerous than normally. The lacunar grooves usually seen at the outer surface of the shaft and filled with osteoclastic cells were scarce and shallower than usual. Thus the cortex appeared smoother and somewhat thicker than is ordinarily the case. The osteocytes of the compacta were dense and the vascular canals were narrow though congested. At the later stages, simultaneously with the progress of absorptive processes in the diaphysis, the periosteal tissue became loosened and contained enlarged capillaries. It proliferated and participated in the destruction of the bony shaft in its entire length, but particularly at the lateral protuberances, a condition not observed in growing mice similarly treated. The compacta became fibrillated and softened and contained enlarged bone cells, numerous, greatly enlarged capillaries, and wide spaces frequently filled with marrow and connective tissue. Thus, vascular and cellular absorption, acting from the outside of the shaft as well as from

the inside, led to a pronounced thinning of the bony shaft. In advanced cases the cortex could appear almost as thin as paper, especially near the lateral protuberances, where the occurrence of fractures sometimes seemed imminent; moreover, bone marrow or fibrous tissue actually invaded and dissolved the compacta, a condition not found in normal old mice.

(c) *Joint*. The cartilage of the articular covering hyalinized and ossified increasingly under the influence of estrogen. Although in some cases, in particular after prolonged administration of estrogen, hyperplasia and hypertrophic changes of the cartilage cells may have occurred, the simultaneous hyalinization of the matrix seemed to prevent these changes from making progress, as was evidenced by the envelopment of accumulations of hypertrophic or degenerated cells in hyalinized ground substance. Thus both incidence and severity of arthropathic lesions, occurring in normal old mice, were greatly reduced subsequent to the administration of estrogen.

III. Older mice

Normal animals. The progress of skeletal ageing found in normal mice up to the age of 19 months has been described in the preceding part II. Since the oldest animals of this group had reached the age of 2 years at the end of the experiment, it may be stated that, in normal mice, during the second half of the second year of life, sclerosis, ossification and perforation of the epiphyseal plate make, as a rule, some progress, but they do not proceed far beyond the stages reached during the preceding months.

The cortex of the long bones gradually decreases in thickness, owing to a decrease of the appositional and an increase of the resorptive processes acting on its surface. The shaft remains, however, intact and consists of a dense bony substance which contains small osteocytes.

Effects of estrogen in older mice, aged 12 to 21 months
at the beginning of the experiment

In growing mice the epiphyseal cartilage underwent marked calcification, sclerosis and destruction subsequent to the ad-

ministration of only 800 RU of estrogen during a period of 1 month.

(a) *Epiphyseal disk*. In old mice, a total amount of 400 to 1600 RU of estrogen, administered in doses of 200 RU weekly for a period of from 2 to 8 weeks, failed to exert a conspicuous effect on the epiphyseal plate, which, according to the age of the animal at the beginning of the treatment, had then already been in an advanced stage of sclerosis and ossification. However, total amounts of 3000 to 7400 RU, injected in weekly doses of 200 RU over a period of from 3 to 8 months, caused an intensification of sclerosis, calcification and ossification of the cartilage, and, simultaneously, an increased absorption of the ossified plate. These absorptive processes led to a general thinning of the epiphyseal plate rather than to an increase in number or extent of localized perforations of the disk. But in some animals, which had been under treatment for 4 and more months, ossification of cartilage and epiphyseo-diaphyseal union were far progressed, farther than in the majority of normal old mice; but the degree of epiphyseo-diaphyseal union even then did usually not exceed the maximum observable under normal conditions.

(b) *Metaphysis and diaphysis*. Administration of 200 RU of estrogen weekly, for periods of from 2 to 6 weeks, during which a total dose of 400 to 1200 RU was given, caused a congestion of the capillaries of the bone marrow, but did not lead to the production of an interlaced bony network in the metaphysis (fig. 2), as was seen in growing mice treated similarly. Megakaryocytes, blood-forming and reticulum cells proliferated by way of mitosis and an increased amount of connective tissue was found in the neighborhood of the pre-existing bony structures of the shaft, of the ossified epiphyseal plate, and of the few persisting spicules. The megakaryocytes often reached considerable size, coalesced and disintegrated. The number of osteoblasts was not increased.

The bony cortex, which had been thick and solid in growing animals at this stage of the experiment, contained great numbers of enlarged blood vessels; they were congested and could

be traced into the loosened periosteal tissue. The bony substance became more and more softened and fibrillar, and the osteocytes enlarged. At the end of this period, the number and width of the blood vessels in the bony shaft had considerably increased and they could be accompanied by bone marrow or connective tissue. Thus, inside the osseous shaft areas of connective tissue were found, which in most cases made connections with the stroma of the bone marrow (fig. 5).

After injections of estrogen for 2 and more months (2000 to 7400 RU) an increasing accumulation of epithelioid cells became noticeable in the subepiphyseal zone. These cells deposited bone, causing a thickening of the ossified epiphyseal plate and subsequently an encroachment upon the adjoining part of the bony shaft, where, in the corners between the shaft and the epiphyseal plate, a loose bony network began to form (fig. 3). Subsequently, an interlaced meshwork crept along the inner surface of the shaft and also extended into the diaphyseal cavity, partly occluding it.

While at the height of these changes there could be present a considerable amount of newly formed bone, which could extend deep into the marrow cavity, there was no dense, continuous network found, such as may be seen in the younger mice under corresponding experimental conditions. Instead, the osseous structures, even if they were fairly thick, were more disconnected and irregularly arranged, and they were separated from each other by a more or less fibrous connective tissue, which had been present already at the earlier stages and which since had increased in amount, apparently destroying the newly formed bone.

Owing to the continuation of both increased absorption and production of bone, a vascularized, partly osteoid, partly fibrous tissue had replaced the ordinarily hemopoietic marrow. These changes were encountered in seven of ten cases. In three cases, the only changes in the diaphyseal cavity consisted of a slight fibrosis of the marrow.

As stated before, the shaft became secondarily involved in the processes of apposition, as the newly formed bone crept

downward from the epiphyseal plate. But also the resorptive processes, absent in young mice but noted in older mice already at the earlier stages of treatment with estrogen, had made progress, and they caused an irregular invasion of the shaft by vessels and connective tissue, leading to a marked thinning of the cortex (fig. 6). However, if larger areas of connective tissue appeared in the bony shaft, small osseous spurs, protruding into the marrow cavity, were found very close to the former. These spurs had no connection with the bone growing downward from the epiphyseal plate, but they seemed to be bound up with the resorptive processes inside the shaft. They could be eventually incorporated into the irregular bony network and later be destroyed by the proliferating connective tissue.

(c) *Joint.* As was the case in the growing mice treated with estrogen, hyalinization and sclerosis of the articular cartilage was increased over the normal, and this effect apparently counteracted the hypertrophic and hyperplastic or degenerative arthropathic changes observable in old untreated animals.

THE SIGNIFICANCE OF STRAIN AND SEX IN THE RESPONSE OF THE SKELETAL TISSUES TO ESTROGEN

In the present experiments the response of the skeletal tissues to the administration of estrogen took a similar course in C₅₇, CBA, New Buffalo and Old Buffalo mice. Mice of strain C₃H reacted less intensely than the former. A middle-aged C₃H mouse which had received 1500 RU of estrogen for 2½ months, showed a hardly noticeable increase in the amount of subepiphyseal bone, and the condition was comparable to that seen in a middle-aged C₅₇ mouse which had received only 400 RU of estrogen during a period of ½ month. After administration of about 4000 RU of estrogen during 4 months, the formation of the subepiphyseal bony network, as well as the absorptive processes at the shaft, had progressed so little in middle-aged C₃H mice, that they were comparable to the reaction of an Old Buffalo mouse which had received only 1500 RU for a period of 3 months.

Since, according to our previous findings ('41 b), growing C₃H mice likewise exhibited a lesser responsiveness to the estrogen than did mice of strains C₅₇ and CBA, it may be concluded that the relative susceptibility of a strain to hormone action, as compared with that of other strains, does not change with increasing age of the animal. This strain characteristic seems to remain constant.

This observation apparently holds good also for D mice. Among the strains investigated formerly, D mice treated with estrogen had shown the greatest tendency to resorptive processes and the removal of the excess bone had set in earlier than in the other strains. This tendency to vigorous resorption of bone subsequent to the administration of estrogen was likewise present and even more accentuated in middle-aged D mice injected with estrogen. As compared with conditions in middle-aged C₅₇ mice, in D mice the destruction of bone by connective tissue was greater in the shaft as well as in the diaphyseal bony network.

As in growing mice treated with estrogen, so also in both the adult and the old animals the skeletal tissues of males seemed to react more strongly to the administration of estrogen than those of females, as was indicated by the greater increase in appositional and resorptive processes of bone in the male.

Similarly, virgin mice seemed to respond more readily to the estrogen than did breeders. In a breeding CBA mouse, 16 months old at the beginning of treatment and having received a total dose of 2600 rat units over a period of 3 months, resorptive processes were noticeable and apposition of bone in the metaphysis was slight, whereas in a corresponding virgin both processes of bone formation and absorption of bone had been very marked. In two other pairs treated for 4 months, similar differences were noted between the conditions in virgins and in breeders.

COMPARISON OF THE SKELETAL CHANGES PRODUCED BY ESTROGEN IN THE VARIOUS AGE GROUPS

From the present material and from that used in a former investigation, thirty-three pairs of mice were selected; the

partners of each pair belonged to the same strain and had received the same dose of estrogen during the same period of time, but they differed from each other in the age at which the treatment with estrogen was begun. Nineteen of these pairs were of identical sex.

For the purpose of this comparison, the changes taking place in the epiphyseal cartilage are of secondary significance, since in old animals the effect of the estrogen on the cartilage is less conspicuous than that on the bone and bone marrow. Therefore, the findings in the latter tissues served primarily as a basis for comparison.

In C_{57} mice, 300 to 900 RU of estrogen in oil, injected for 2 to 4 weeks, from the age of 4 to 6 weeks on, caused a marked inhibition of the removal of the subepiphyseal spicules, which occurs normally during the course of growth of the bones. The amount of osseous substance in the metaphysis had greatly increased as compared with the normal. In C_{57} mice, which had received the same amount of estrogen over the same length of time, but which were 6 months old at the beginning of the experiment, there was only a very slight thickening of the ossified epiphyseal plate observable, and in corresponding C_{57} mice, 18 months old at the beginning of the treatment, there was no noticeable effect on the bony structures inside the diaphyseal cavity. However, in the adult, and even more so in the old mice, treated with estrogen the capillaries of the bone marrow and of the vessels perforating the bony shaft were congested and enlarged, a condition not seen in corresponding young mice. Moreover, there was present in the older animals a greater increase in the amount and density of connective tissue of the bone marrow, which was particularly accentuated in the metaphysis.

Subsequent to the administration of about 1500 RU of estrogen over a period of 2 and more months, in young C_{57} and CBA mice a dense interlaced network of bone was found in the diaphyseal cavity, whereas in a corresponding CBA mouse, 16 months old at the beginning of the injections, a bony meshwork had just begun to develop underneath the epiphyseal

plate. Furthermore, in this case enlarged vessels and proliferating connective tissue had invaded and partly dissolved the compacta of the shaft, which was dense and solid in young mice treated with estrogen. An adult mouse which had received the same dose of estrogen for the same length of time, took an intermediate position between the growing and the old animals. The amount of bone and the extent of the osseous network were greater than in the old animal, but they were smaller and the network was looser than in the young animal. In the shaft the vessels were enlarged, as was the case in the old mice, but there was no invasion by connective tissue, as had been observed in the latter.

After injection of larger doses of estrogen, corresponding differences in the response of the skeletal tissues in the three age groups could be established. In the growing animal, absorptive processes had increased as compared with the earlier stages; but after injection of about 3000 RU of estrogen for 4 months, two CBA mice, a half month old at the beginning of the treatment, still showed a fairly dense diffuse network of bone in the diaphyseal cavity and no changes in the compacta of the shaft, whereas, in two CBA mice, 16 months old at the beginning of the treatment, there was found only a loose bony network, restricted to the immediate neighborhood of the epiphyseal disk, and destruction of the shaft by fibrous tissue had progressed a great deal.

Although C_3H mice do not respond so vigorously to the administration of estrogen as CBA or C_{57} mice, the reaction in the different age groups differed in the same direction as in the latter strains. In a C_3H male mouse, 7 months old at the beginning of the injections and injected with 1500 RU of estrogen for $2\frac{1}{2}$ months, resorptive processes counteracted the new formation of bone, whereas in a C_3H mouse which had been 1 month old when the treatment was begun, the resorptive processes were inhibited and the increase in the amount of bone was definitely greater than in the older animal. In the latter, moreover, the vascularization of the shaft was considerably increased as compared with the younger mouse.

Three thousand rat units of estrogen had caused, in a growing C_3H mouse, a pronounced thickening of the spicules, but in an adult C_3H mouse there was only slightly more bone present, as was evidenced by the appearance of a small, loose, bony metaphyseal network.

In adult D mice, which had received about 1500 RU of estrogen for 2 months, less bone was present and the resorption of the trabeculae had progressed much farther than in young D mice injected with the same amount of estrogen for the same length of time.

The following table may, in a schematic way, demonstrate the correlation between apposition and resorption of bone in the various age groups.

TABLE 3

	APPOSITION OF BONE	RESORPTION OF BONE
Young mice:	Greatly increased. Accelerated.	Greatly decreased. Delayed.
Adult mice:	Moderately increased. Somewhat delayed.	Moderately increased. Somewhat accelerated.
Older mice:	Slightly increased. Greatly delayed.	Greatly increased. Accelerated.

DISCUSSION

In mice, the effect of estrogen on the skeletal tissues changes with increasing age of the animal. This age effect may be explained partly by the fact that the structure of these tissues changes as the animal grows older. In young mice, the epiphyseal cartilage is growing and is being constantly replaced by bone, which in turn is absorbed by bone marrow. In adult and old mice, on the other hand, the epiphyseal cartilage has become inactive, uniform in structure, and more or less ossified; the metaphyseal trabeculae have been completely or almost completely absorbed. Since these two tissues largely determine the response to estrogen in young animals, the replacement of the cartilage by other tissues, and the absence of the trabeculae would necessarily alter the response to estrogen in older animals. In young mice, an early effect of estrogen

manifested itself in an inhibition of absorption of trabeculae, which persisted longer than usual and thus provided a substratum for the later changes.

However, the age changes in the histological substratum could only partly account for the differences in reactions of the skeletal tissues to stimulation by estrogen in the various age groups. Whereas the character of the growth zones changes with increasing age, the compact bone of the shaft, the bone marrow and the periosteal tissue remain more constant at the various ages. In normal animals there exists a permanent interaction between these tissues, and a balance between appositional and resorptive processes determines the amount of bone present at any given age. This balance between apposition and resorption is greatly interfered with by the action of estrogen in growing and older mice, but the equilibrium is disturbed in a different way in the various age groups. In the growing animal, the balance between apposition and resorption of bone is disturbed by a decrease in resorptive and an increase in appositional processes, both these effects causing a great excess of bone in the diaphyseal cavity. A restoration of an approximately normal balance is accomplished subsequently by the resumption and increasing intensity of resorptive processes.

In older mice, the disturbance of the balance manifests itself as follows: (1) The resorptive processes which were inhibited during the early stages of administration of estrogen in young animals are not inhibited in adult or in old animals; they may even be intensified in both the older groups, as indicated by the increased vascularization of the shaft. (2) At a subsequent stage apposition of bone was noted in the adult group, which gradually increased in amount but was interfered with by the simultaneously increasing processes of absorption, which apparently prevented the formation of such a dense bony network as was seen in the young animals. The bony shaft underwent increased vascularization and thinning as compared with the conditions in growing animals. In old mice, the tendency to deposition of bone in the diaphyseal

cavity was even less strong than in the adult group; the increase in the amount of bone was smaller and it began to appear later than in the latter; resorption of the shaft was accomplished not only by increased vascularization, but also by invasion of connective tissue. (3) While processes of absorption were only beginning in growing animals at later stages, in old mice destruction of the loose bony network by proliferating connective tissue and capillaries was already far advanced; widespread absorption of the bony shaft had led to a partial destruction, softening and porosis, conditions not found in young mice similarly treated.

Thus, in older mice, the disturbance of the balance between formation and removal of bone subsequent to the administration of estrogen had shifted in favor of resorption. This shift manifested itself both as to time and to intensity, and it was the more pronounced the greater the age of the animal at the beginning of the treatment.

In a recent publication which appeared after our earlier paper ('41 b) had gone to press, and while the present paper was nearing completion, Day and Follis ('41) reported on the effect of estrogen on the epiphyseal plates and the shaft of the long bones of rats of various age groups. They found in young rats, subsequent to administration of estrogen, an inhibition of absorption of the bony spicules, a condition which we likewise observed in young mice and rats. In middle-aged and old rats, these investigators noted a diminution of this effect. The failure of the old rats, used by Day and Follis, to develop increased resorptive processes may be due to the relatively short duration of their experiment (37 days); it is also possible that the rats used were still too young to show the age effect, although they had reached the age of 2 years at the beginning of the experiment; or that rats in general, and the strains used in particular, may show a lower responsiveness to estrogen, such as seen in mice of strain C₃H and in guinea pigs.

Previously, we raised the question as to whether the resumption of absorption under the prolonged influence of estrogen on the skeletal tissues of growing mice, might be due to an adaptation of the organism to the hormone, or whether it might be related to the advancing age of the animal and to an ensuing change in the mode of reaction manifesting itself in a greater tendency to absorption of bone. The present findings provide evidence for such a tendency. Thus a similarity exists between normal mice and mice treated with estrogen, inasmuch as in the skeletal tissues of both, resorptive processes become more accentuated with advancing age. However, in the animals treated with estrogen, the resorption of bone is so greatly intensified as compared with the normal condition, that the resulting softening and porosis of bone should, at least partly, be due to the action of estrogen.

The delayed and decreased apposition of bone in the old animals might be considered as due to a general increase in resistance to the hormone in older animals. However, the early response of the bone marrow and of the shaft indicates an early reaction to estrogen also in older mice. Since the inhibition of resorptive processes is lacking in old animals, the excess of bone seen under the influence of estrogen is probably due to processes of apposition. The latter may be initiated by an accumulation of osteoblastic epithelioid cells around various places in the metaphyseal tissue, the density of which was increased by processes of fibrosis. The increase of dense connective tissue around the endarteries in the metaphysis may perhaps create a state of malnutrition favorable to bone formation.

Besides the hormonal effects, local conditions may influence the processes of bone formation. This is suggested by the changes seen at the shaft, where processes of increased vascularization and softening are present at the early stages without indication of increased apposition of bone. At later stages, however, when connective tissue has replaced the compact

bone, there occurs in contact with such areas an irregular formation of bone. This bone production could be due to the action of estrogen, but it may, as well, be due to a non-specific reaction, comparable to other reactions observed in the osseous system under various pathological conditions, in which increased resorption and apposition of bone frequently go hand in hand.

SUMMARY

Estrogen interferences with both apposition and resorption of bone. However, the mode of this interference is influenced by the age of the animal at the beginning of the treatment. In young growing mice, the equilibrium between apposition and resorption of bone is such that resorptive processes are diminished and appositional processes promoted. In these animals, at early stages, estrogen produces an excessive amount of bone in the marrow cavity, which subsequently is removed on account of an increase in absorptive processes. In older mice, the resorptive processes are present from early stages of the experiment on and subsequently increase. The processes of apposition become manifest only at a later date and are decreased. Excess of bone is, therefore, found in the older animals only at later stages, and it is less in amount than in the growing animals. In addition, in old animals intensified resorptive processes cause a softening and porosis of the shaft of the long bones.

As in growing mice, so also in older mice ageing processes of the inactive epiphyseal cartilage may be accelerated under the influence of estrogen, but the degree of epiphyseo-diaphyseal union ultimately reached in the latter, does as a rule not exceed the maximum observable in normal old mice. The incidence and severity of arthropathic changes in older mice treated with estrogen are decreased as compared with conditions in untreated old mice, but they seem to be greater in mice in which the treatment was begun at an older age than in those which received the estrogen injections from an early age on.

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PLATE 1

EXPLANATION OF FIGURES

1 Section through the metaphysis of a C₅₇ virgin mouse which, starting at the age of 6 months, had received injections of 200 RU of estrogen weekly for 4 weeks. Bone formation in the metaphysis, creeping along the inner surface of the shaft. Congested capillaries in the metaphysis and in the shaft. Magnification $\times 100$.

2 Section through the metaphysis of a male CBA mouse which, starting at the age of 17 months, had received injections of 200 RU of estrogen weekly for 4 weeks. No bone formation in the metaphysis. Epiphyseal zone consists of remnants of hyalinized and calcified cartilage. Same magnification as in figure 1.

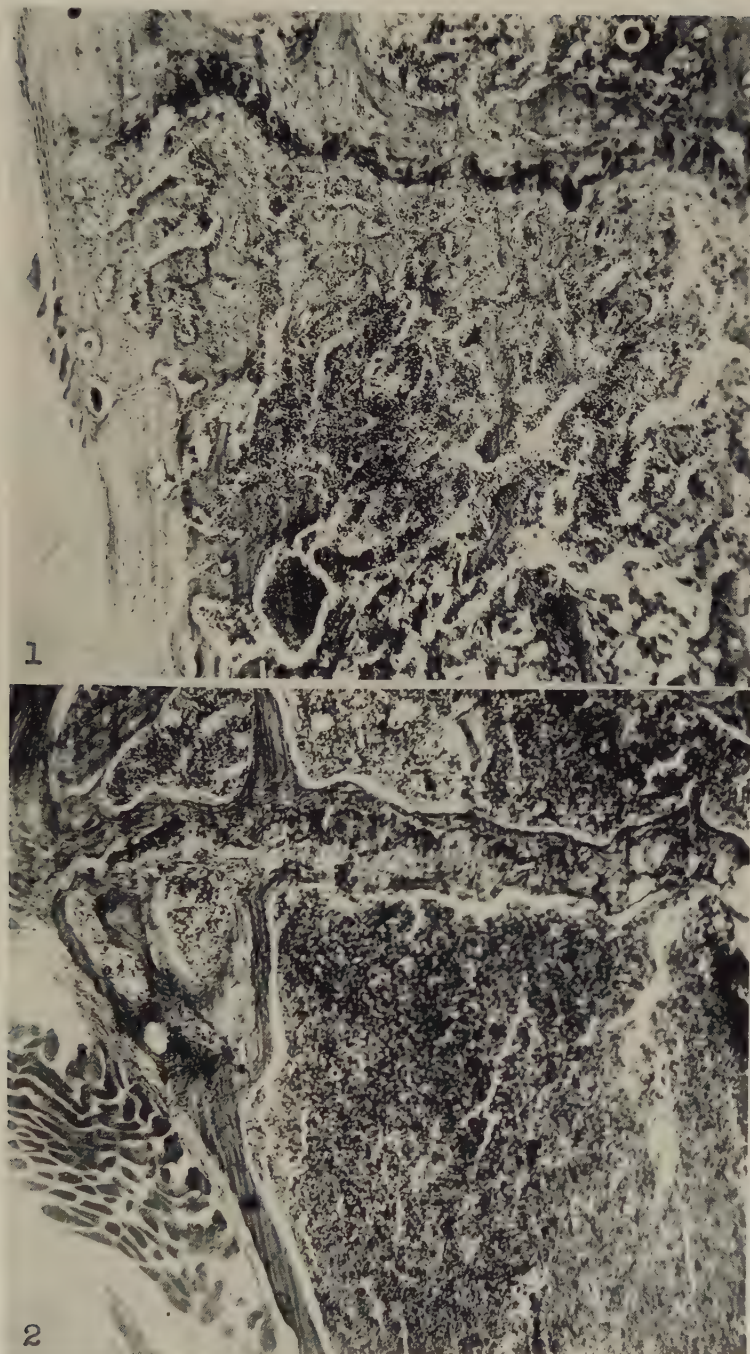


PLATE 2

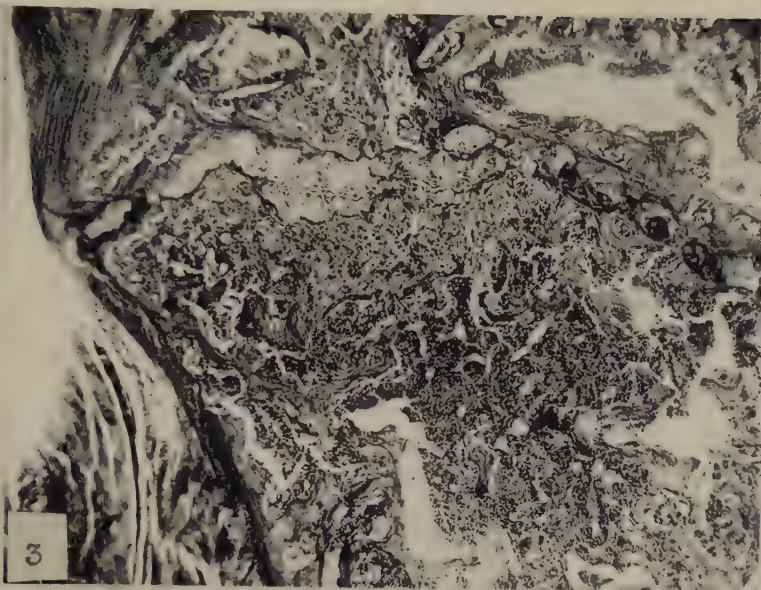
EXPLANATION OF FIGURES

3 Section through the metaphysis of a CBA female breeding mouse which, starting at the age of 14 months, had received injections of 200 RU of estrogen weekly for 5 months. In the subepiphyseal layer formation of a loose bony network, which is absorbed by connective tissue. Some bony spurs at the inner surface of the thinned shaft. Same magnification as in figure 1.

4 Section through the shaft of the tibia of a normal virgin CBA mouse, 16 months of age. Bony compacta solid, consisting of small osteocytes and dense ground substance. Magnification $\times 150$.

5 Section through the shaft of the tibia of a virgin CBA mouse which, starting at the age of 15 months, had received injections of 200 RU of estrogen weekly for a period of 6 weeks. Bony compacta softened and laminated, containing enlarged spaces filled with capillaries and bone marrow. Same magnification as figure 4.

6 Section through the shaft of the tibia of a virgin C₅₇ mouse which starting at the age of 12 months, had received 200 RU of estrogen weekly for a period of 4 months. Bony compacta greatly thinned out and replaced by osteoid connective tissue. Same magnification as in figure 4.



THE ARTERIAL NETWORK SUPPLYING THE DORSUM OF THE FOOT¹

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THREE FIGURES

The standard textbooks of gross anatomy are in good general agreement concerning the description of the dorsalis pedis artery and its branches which supply the dorsum of the foot. However, many variations have been described and it is a common experience in the dissecting room to have difficulty in demonstrating the textbook picture of these vessels. The present study was undertaken to add to our knowledge concerning the degree of variability of the arterial supply of the dorsum of the foot and to attempt to find some description or series of descriptions which would apply to as many individuals as possible.

The material upon which this study was carried out consisted of both feet of each of 100 cadavers consecutively placed in the dissection room for student use. In this group, there were fifty-six white males, five white females, thirty-four negro males and five negro females. The recorded ages ranged from 21 to 83 years.

In every instance, the arterial network was dissected out in detail by the author and a sketch was made of each network in its relationship to the bony framework of the foot.

¹ A preliminary report on a portion of the material covered in this paper was presented at the Fifty-fourth Annual Session of the American Association of Anatomists, April 14 to 16, 1938, Pittsburgh, Pa.

THE ARRANGEMENT CONSIDERED AS A WHOLE

In describing the results of this investigation, attention will be given first to the arrangement of arteries as a whole and then to the individual vessels.

The standard description of a dorsalis pedis artery first giving off lateral and medial tarsal branches, next, at about the level of the first tarsometatarsal joint, an arcuate artery with second, third and fourth dorsal metatarsal branches, and then a first dorsal metatarsal artery, with each of the dorsal metatarsal arteries dividing into two dorsal digitals, was found in only eleven of the 200 feet dissected. However there was no other arrangement which was present in more than nine instances. Of three such, one differed from the "standard" principally in that the arcuate arose at about the level of the cuneonavicular articulation; another differed principally in that the fourth dorsal metatarsal did not give off dorsal digitals, these coming instead from the plantar aspect; and the third differed in that the fourth dorsal metatarsal was absent, the dorsal digitals coming again from the plantar aspect.

In addition to the four types just described, there were some seventy to eighty others (depending on the interpretation) present in from one to eight instances each, differing from one another in varying degrees. No attempt will be made to describe, or even tabulate all of these individual arrangements, but some idea concerning them can be gained by grouping them in the following manner:

- A. Arcuate artery arising at about the level of the first tarsometatarsal joint and giving rise to (1) the second, third and fourth dorsal metatarsals; (2) the second and third dorsal metatarsals; and (3) only the second dorsal metatarsal.
- B. Arcuate artery arising at about the level of the naviculocuneiform joint. (Subgroups 1, 2 and 3, the same as under A.)
- C. No arcuate artery present.
- D. Dorsalis pedis artery practically absent.

Figure 1a can be taken as a composite for group A, 1b for group B, 1c for group C and 1d for group D if it is realized that in those instances in which the second, third or fourth dorsal metatarsal artery does not come from the arcuate, it may come from either the lateral tarsal or the posterior perforating artery and that any or all of the dorsal digital arteries may come from either a dorsal metatarsal artery or from the plantar aspect.

The number of times the groups just described were found in the 200 feet dissected is as follows:

Group A—70 (35%)

A1, 33 (16.5%); A2, 18 (9%); A3, 19 (9.5%)

Group B—38 (19%)

B1, 18 (9%); B2, 13 (6.5%); B3, 7 (3.5%)

Group C—68 (34%)

Group D—24 (12%)

In the course of the dissections it became apparent that there was a pattern (fig. 1e) upon which approximately 98% of the specimens could be superimposed, and that one could demonstrate an arterial network in practically every instance which more or less completely represented that pattern; the individual appearance depending on which vessels were of significant caliber.

ARTERIES CONSIDERED INDIVIDUALLY

The dorsalis pedis artery. In 147 out of the 200 instances, the part of the arterial network on the dorsum of the foot which is given the name "dorsalis pedis artery" followed the usual textbook description of beginning as a continuation of the anterior tibial artery anterior to the ankle joint and running along a line from a point halfway between the two malleoli to the posterior end of the first intermetatarsal space. In following this line, the artery passes across the talus, navicular and second cuneiform bones and the base of the second metatarsal bone, as shown in figure 1e. Although a majority of the textbooks consulted give the description as above, other ways of designating the arbitrary point of the

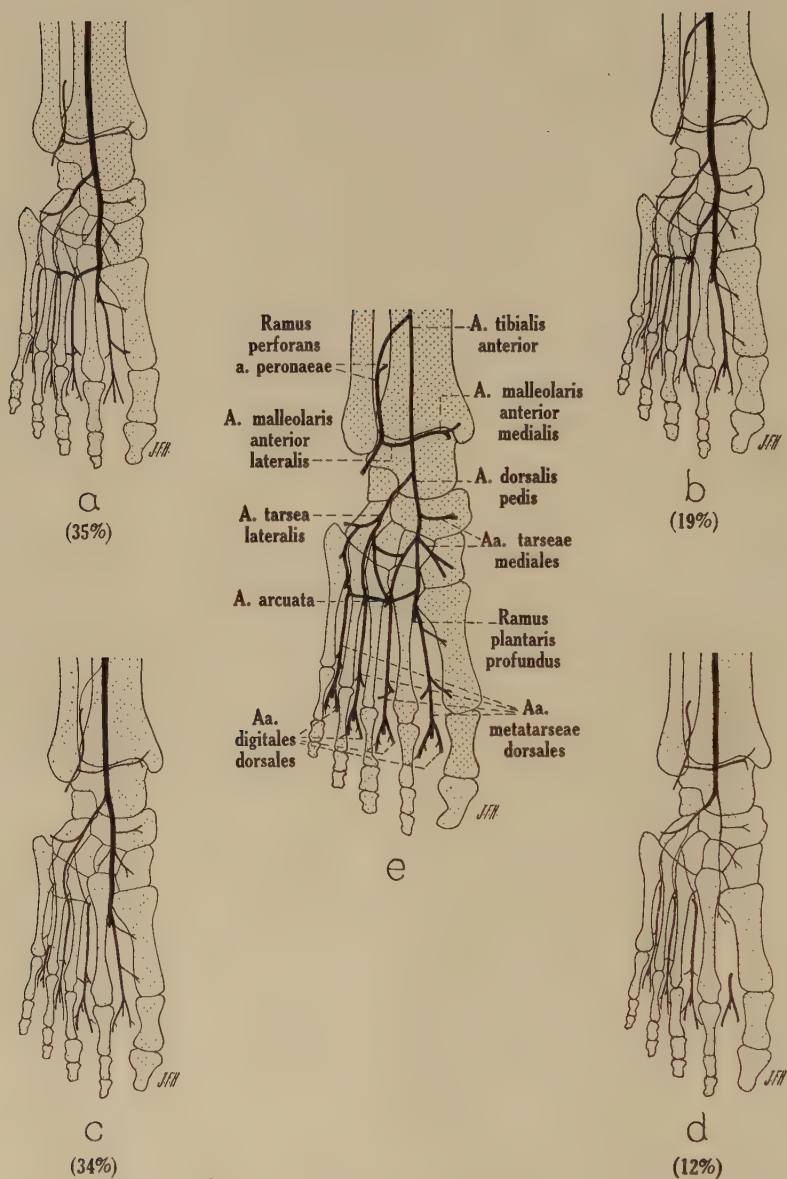


Fig. 1 The arterial network of the dorsum of the foot. a, b, c and d serve as general pictures for the main groups as explained on page 375. The percentage given is for the group represented. e is the pattern of the network. It can be seen that a, b, c and d, can be superimposed on this pattern.

beginning of the dorsalis pedis artery are: "where the anterior tibial artery crosses under the extensor hallucis longus tendon" (Corning, '11; Tillaux, '14; this would be a less definite point as the tendon overlaps the artery for some distance); "as the artery passes under the annular ligament" (Testut, '21); "at the lower border of the frondiform ligament" (Rouvière, '32).

The fifty-three instances in which the dorsalis pedis artery differed from the usual description can be summarized as follows:

Twenty-four cases (12%): artery so reduced in size that one might speak of it as absent (fig. 1d).

Eleven cases (5.5%): artery deviated laterally from usual position.

Seven cases (3.5%): artery deviated medially from usual position.

Six cases (3%): artery began as a continuation of the perforating peroneal artery (fig. 2a).

Three cases (1.5%): lower end of anterior tibial artery in position of perforating peroneal artery (fig. 2b).

One case (.5%): artery arose about equally from anterior tibial and perforating peroneal (fig. 2c).

One case (.5%): artery much smaller than usual but not enough so to be spoken of as absent.

Here and there in the various written descriptions of the dorsalis pedis artery, all of the above variations are referred to, but there is a scarcity of data as to their frequency. To compare with the present finding of 12% of the cases in which the dorsalis pedis might be spoken of as absent, Adachi ('28) reported seven out of 230 feet (3%) in which the dorsalis pedis was so small that it only "reached with a thin end to the first intermetatarsal space," Reich ('34) found ten out of seventy feet (14.2%) in which the dorsalis pedis was small or absent, and Morrison's ('33) data can probably be interpreted as showing five or six out of sixty-two feet (8 or 9%) in which the artery was extremely small. Adachi ('28) states that in his studies (and those of Liu) eighty-

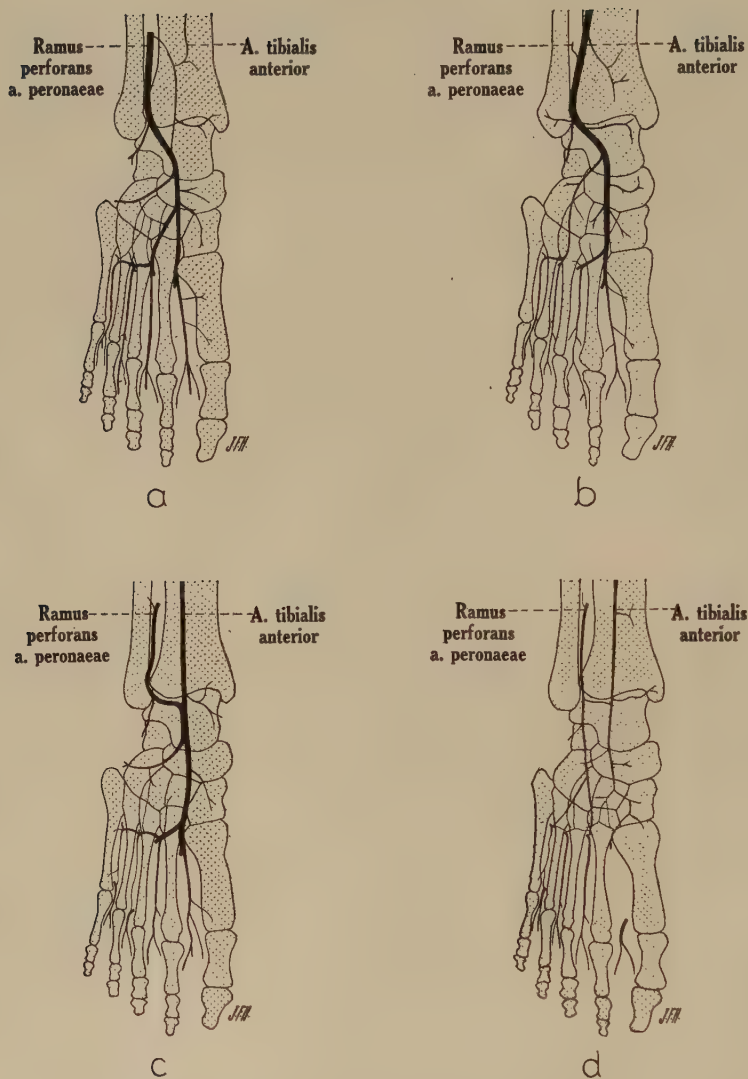


Fig. 2 Additional individual pictures of the arterial network of the dorsum of the foot. a shows the dorsalis pedis artery as a continuation of the perforating branch of the peroneal artery. b shows the lower end of the anterior tibial artery apparently occupying the position of the perforating branch of the peroneal artery. c shows the dorsalis pedis artery coming equally from the anterior tibial artery and the perforating branch of the peroneal artery. d appears to show the lateral tarsal artery as a continuation of the perforating branch of the peroneal artery.

eight out of 1239 feet (7.1%) showed the dorsalis pedis artery to be a continuation of the perforating peroneal, and he contrasts this finding of 7.1% in Japanese with 3.3% in Europeans as reported by several authors. This percentage for Europeans agrees well with the 3% reported in the present investigation.

Piersol ('30) mentions the fact that the dorsalis pedis artery has been observed as a continuation of a branch from the lateral plantar artery coming through the tarsal sinus. This probably refers to a case reported by Hyrtl which Senior ('19) refers to as unique.

The lateral tarsal artery. This artery arose from the dorsalis pedis artery at about the level of the junction of the neck and head of the talus in 116 of the 200 feet. In thirty-nine instances, its point of origin was higher (up to almost as high as the ankle joint, and arising in common with the anterior lateral malleolar artery thirteen times), and in thirty-eight it was lower (down to just below the talonavicular joint). The lateral tarsal artery could not be definitely identified in three feet, it was extremely small in three others and in one, it was apparently a continuation of the perforating branch of the peroneal artery (fig. 2d). Allen (1884), Poirier and Charpey ('12, in the text material but not in the accompanying figure), Deaver ('26) and Gray ('36) give the point of origin of the lateral tarsal artery as over the navicular bone. Other descriptions consulted give the point in a position compatible with the one found most commonly in this study.

The course and bony relationships of the lateral tarsal artery are well shown in figures 1 and 2, as are its anastomoses with the arcuate and anterior lateral malleolar arteries. What are fine anastomotic branches to the arcuate artery in some individual arrangements are the beginnings of the second, third or fourth dorsal metatarsal arteries in others (fig. 1c, third dorsal metatarsal artery).

Adachi ('28) reported that he found the lateral tarsal artery larger than the portion of the dorsalis pedis beyond the point that vessel branched off in 5.2% of 230 feet, which figure he compared with 30% of fifty feet of the newborn re-

ported by Corsey and 17.5% of 200 feet reported by Salvi. Reich ('34) found the lateral tarsal to be the larger in 7% of seventy feet, which is exactly the percentage found in the present series of 200 feet.

The medial tarsal arteries. The medial tarsal branches of the dorsalis pedis artery varied greatly in number and size. Eighty-four out of 200 feet demonstrated two branches of about equal size, one near the middle of the navicular bone and one just below the cuneonavicular articulation. In twenty-six instances, the proximal and in twenty-seven, the distal branch appeared to be present alone. In thirty feet, a short common stem at about the position of the proximal medial tarsal divided into two branches, one running proximally and the other distally. Minute branches, varying in number and running medially from the dorsalis pedis artery, were also present in most instances, either in addition to the ones described above or as the only medial tarsal branches.

The arcuate artery. The part of the arterial network on the dorsum of the foot which is given the name "arcuate artery" was present as a vessel of significant size in only 108 of the 200 feet, and, when present, it varied both as to the point of branching from the dorsalis pedis artery and as to length. In seventy feet it branched off at the level of the first tarsometatarsal joint (fig. 1a) and thirty-eight showed it branching off at the cuneonavicular joint (fig. 1b) or a little below (a little below in four). The arcuate artery may be just long enough to give off the second dorsal metatarsal artery (fig. 2b) or the second and third or the second, third and fourth dorsal metatarsals. The incidence of these variations in length is shown on page 375.

Although stated in many ways, most descriptions give approximately the first tarsometatarsal joint as the level at which the arcuate artery branches from the dorsalis pedis artery. However, Toldt ('26, fig. 1043) shows it branching off at about the level of the cuneonavicular joint and Henle (1868, vol. 111, p. 195, fig. 101), in his figure of the dorsalis

pedis artery and its branches, shows a similar picture, labelling the vessel as "the anterior lateral tarsal artery," but giving "metatarsal artery" as his name for the anterior lateral tarsal artery in a footnote on the preceding page. Adachi ('28) stresses the fact that he thinks Toldt was wrong in calling the artery branching off at the level of the cuneonavicular joint the metatarsal artery (arcuate artery) and that he thinks only the branch coming off the dorsalis pedis artery at the level of the first tarsometatarsal joint should be called the arcuate artery and the one at the level of the cuneonavicular joint should be called the anterior or distal lateral tarsal artery. This seems to be just a matter of interpretation, since that part of the artery extending laterally from the posterior end of the second intermetatarsal space and giving off the second, third and fourth dorsal metatarsal arteries appears to be the same in both instances (figs. 1a, 1b; compare also, Adachi's figs. 169 and 173).

The findings as to the arcuate and distal lateral tarsal arteries reported by Adachi, when interpreted in terms of the grouping used in the present study, give the following results: A1, 13%; A2, 5.5%; A3, 12.1%; B1 3%; B2 1.3%; B3, .4%; that is, group A, 30.6% and group B, 4.7% (compare p. 375). Dubreuil-Chambardel's findings, as reported by Poirier and Charpey ('12), can be interpreted as giving 20-25% in group A and about 3-8% in group B.

The dorsal metatarsal arteries. The first dorsal metatarsal artery branched from the dorsalis pedis artery in 153 of the 200 feet, as in the textbook description. In seventeen feet it came up from the plantar aspect and in six it appeared to come equally from the dorsalis pedis artery and the plantar aspect. The remaining twenty-four feet had no first dorsal metatarsal artery of significant size (figs. 1d and 2d). In passing, it was noted that there was often a small additional dorsal artery in the first intermetatarsal space and that there were variations in the relations of the artery to the interosseous musculature.

The results of the observations on the source of the second, third and fourth dorsal metatarsal arteries can be summarized as follows:

PRINCIPAL SOURCE	DORSAL METATARSAL ARTERIES		
	Second	Third	Fourth
Arcuate artery	99	77	47
Posterior perforating artery (fig. 1d)	67	47	75
Lateral tarsal artery (fig. 1c, third dorsal metatarsal)	11	41	34
Posterior perforating and lateral tarsal artery	9	17	10
Posterior perforating and arcuate artery	4	4	0
(No dorsal metatarsal of significant size)	8	12	28
(Not classified)	2	3	6

It must be realized that the decision as to what is the principal source of a particular vessel is not always easy, since there is usually anastomosis between the arcuate, lateral tarsal, posterior perforating and dorsal metatarsal arteries (fig. 1e) and differences in the size of these vessels may not be great.

Adachi ('28) grouped the dorsal metatarsal arteries as to whether their principal source was "dorsal," "plantar" or about equally "dorsal and plantar" with the following results for a series of 230 feet:

PRINCIPAL SOURCE	DORSAL METATARSAL ARTERIES			
	First	Second	Third	Fourth
Dorsal	186	83	82	78
Plantar	44	130	131	146
Dorsal and plantar	0	17	17	6

If the dorsal metatarsal arteries in the present series of 200 feet are grouped in a similar fashion, the following results are obtained:

PRINCIPAL SOURCE	DORSAL METATARSAL ARTERIES			
	First	Second	Third	Fourth
Dorsal	153	110	118	81
Plantar	17	67	46	75
Dorsal and plantar	6	13	21	10
(Not present or not classified)	24	10	15	34

Whether these results indicate a real difference between Japanese and American feet or only a difference in interpretation of the arterial pictures is difficult to decide.

The mode of termination of the dorsal metatarsal arteries will be considered in the description of the anterior perforating arteries.

The dorsal digital arteries. These arteries branch off near the metatarsophalangeal joint from: (1) the dorsal metatarsal artery; (2) an artery which comes up from the plantar metatarsal artery through the distal part of the intermetatarsal space to occupy the position of the terminal part of the dorsal metatarsal artery (fig. 1c, fourth intermetatarsal space; 2c, third and fourth spaces); (3) the terminal part of the dorsal metatarsal artery contributed to equally by the artery coming up through the distal part of the intermetatarsal space. The following tabulation shows the incidence of each of the above conditions:

CONDITION	DORSAL DIGITAL ARTERIES FOR DIGITS			
	1 and 2	2 and 3	3 and 4	4 and 5
(1)	165	178	158	78
(2)	21	17	31	107
(3)	0	4	7	5
(Not classified)	14	1	4	10

More detail in regard to the digital arteries will be given under the heading "anterior perforating arteries".

The posterior perforating arteries. Although the record on the posterior perforating arteries is not quite complete it can be safely stated that they were not absent in more than 3-5% of the instances. They were found to pass through the posterior end of the intermetatarsal spaces and to connect with the dorsal metatarsal arteries at or near the arcuate artery (figs. 1a and 1b), or with the continuations of branches of the lateral tarsal artery as dorsal metatarsal arteries (fig. 1c, third intermetatarsal space). The incidence of the posterior perforating artery as the principal source of the dorsal metatarsal artery is given in the tabulation on page 382.

The anterior perforating arteries. Most of the written descriptions give the anterior perforatings as located in the anterior ends of the intermetatarsal or interosseous spaces and most of the figures in which they are labelled show them

anterior to the heads of the metatarsal bones. There are anastomoses between the dorsal and plantar arteries in both of these positions (fig. 1e).

The vessels in the anterior ends of the intermetatarsal spaces (fig. 1e) are the ones which apparently become the source of the vessels occupying the position of the terminal part of the dorsal metatarsal artery and giving rise to dorsal digital arteries (p. 383). In 65-75% of the remaining instances, particularly in the second, third and fourth spaces, there were two quite small arteries connecting the dorsal and plantar metatarsals, one close to the junction of the head and shaft of the bone at each side of the space. The two arteries in the second space more often than not had a common stem from the dorsal metatarsal artery.

The description of the anastomoses between the dorsal and plantar arteries anterior to the heads of the metatarsal bones must include the termination of the plantar metatarsal arteries. In about 20-30% of the instances the dorsal metatarsal artery, after giving off the dorsal digitals, appeared to continue in a plantar direction near the web of the toes to help form the plantar digital arteries, in one of the ways indicated in figure 3 a, b, c. This continuation of the dorsal metatarsal artery seems to be the vessel which is often labelled as the anterior perforating artery. In most of the other instances the dorsal metatarsal artery appeared to end by dividing into the two dorsal digital arteries and many times there was an anastomotic branch (fig. 3d) between the dorsal digital artery and the plantar digital artery in relation to the shaft of the first phalanx.

The anterior medial malleolar artery. It was not possible to decide in all cases on any one artery which should be called the anterior medial malleolar artery but in 55-60% of the feet that artery was observed as branching from the dorsalis pedis artery very near its beginning, that is, from the stem artery just below the ankle joint. As Adachi ('28), whose findings are similar, has pointed out, this does not agree with the standard textbook description, which gives

the anterior medial malleolar artery as a branch of the anterior tibial artery. In some instances (about 8%), an artery going to the distribution of the anterior medial malleolar artery branched from the anterior tibial artery 2–3 cm. above the ankle joint; in other instances (about 13–15%), one branched off just above the ankle joint and in still others (about 3–4%), 1 cm. or so below that joint.

The anterior lateral malleolar artery. Since, in 60 to 65% of the feet, the point of origin of the anterior lateral malleolar

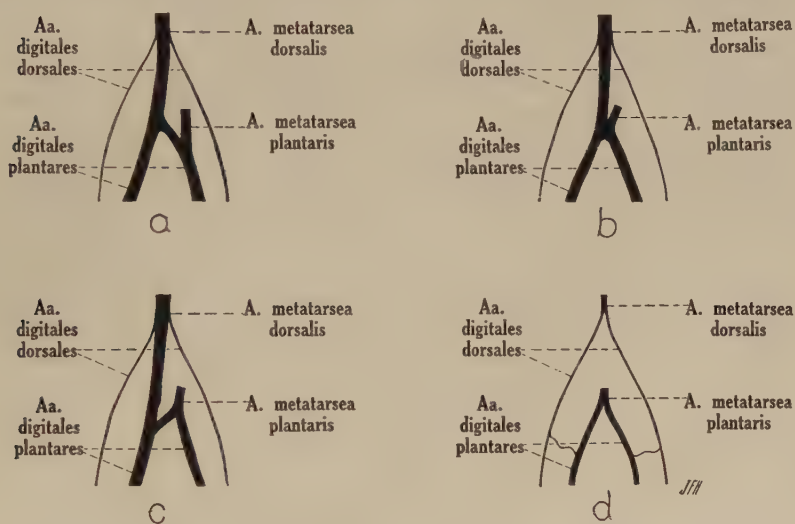


Fig. 3 Anastomoses between the dorsal and plantar arteries in the interdigital spaces. a, b and c show examples of the ways in which the continuation of the dorsal metatarsal artery may contribute to the plantar digital arteries. d shows a connection between dorsal and plantar digital arteries.

artery was at or a millimeter or two below the commonest point of origin of the anterior medial malleolar, it also should probably be listed as a branch of the dorsalis pedis. This artery arose at a lower point in about 10% and at, or just above, the ankle joint in about 3% of the instances.

The perforating branch of the peroneal artery. The usual textbook description of the perforating branch of the peroneal artery as piercing the distal part of the interosseous mem-

brane between the tibia and fibula and coursing distally in front of the inferior tibiofibular joint to anastomose with the anterior lateral malleolar artery was found in about 47% of the feet. In addition, another vessel, a previous description of which has not been found, was present in about 50% of the feet as a vessel of significant size. It branches from the anterior tibial artery about 5 cm. above the ankle joint (figs. 1b, c) and runs inferiorly and laterally to the point at which the perforating branch of the peroneal artery comes through the interosseous membrane to either contribute to the perforating branch of the peroneal as a smaller vessel (about 13%; fig. 1c), join the perforating branch as a vessel of equal size (about 20%), or to be the chief source of an artery occupying the position of the perforating branch of the peroneal artery (about 17%, fig. 1b). Perhaps this vessel is made use of when the lower end of the anterior tibial artery occupies the position of the perforating branch of the peroneal artery as it was found to do in 1.5% of the feet (fig. 2b). As indicated under the description of the dorsalis pedis artery, the perforating branch of the peroneal artery continued as the dorsalis pedis artery in 3% (fig. 2a) and contributed about equally with the anterior tibial to the dorsalis pedis in .5% (fig. 2c). It appeared to continue as the lateral tarsal artery in .5% (fig. 2d).

The artery of the tarsal sinus. This artery, which has been described as a branch from either the dorsalis pedis artery (about 50%, Adachi, '28), the lateral tarsal artery (about 30%, Adachi, '28), the perforating branch of the peroneal artery, or the anterior lateral malleolar artery and as coursing through the tarsal sinus to anastomose with a branch from either the lateral plantar artery, or the medial plantar artery (Poirier and Charpey, '12), was not specifically searched for in the course of these dissections. There was one instance in which a small branch from near the beginning of the lateral tarsal artery called attention to itself because it appeared to be entering the tarsal sinus without breaking up into fine

branches in the adipose tissue as the vessels to this region usually appeared to do.

RACIAL DIFFERENCES

Although the numbers involved are not large enough to warrant an extensive analysis of the data in search of differences between negro and white, there is an indication in the records that perhaps the arterial supply of the dorsum of the foot in the negro, more closely approximates the standard textbook picture than it does in the white; that is, six of the eleven instances (54.5%) in which the textbook picture was present were in negroes and only seventy-eight of the 200 feet (39%) were negro (thirty-nine of the 100 cadavers). Also, if one compares the percentage of the feet of negro males which fall into each of the four main groups (p. 374) with similar percentages for the feet of white males, as is done in the following tabulation, it will be seen that the figures are definitely higher for the negro in those groups (A and B) which can be thought of as more closely approaching the textbook picture and lower in those groups (C and D) less like that picture:

	GROUP A	GROUP B	GROUP C	GROUP D
	%	%	%	%
Feet of negro males	51.4	30.8	13.2	4.2
Feet of white males	25.8	9.8	45.5	18.7

BILATERAL SYMMETRY

Of the 100 cadavers, there were only four in which both feet gave arterial pictures which were exactly alike when all of the details were considered. Only two of the eleven textbook pictures were bilateral. These facts, however, seem to give a wrong impression of the degree of bilateral similarity, as shown by the subsequent data. In sixty-five of the 100 cadavers both feet gave pictures which fell into the same main group (A, B, C or D), in six cadavers both feet gave pictures which were very similar although not in the same main group, in eighteen there was some degree of similarity

and in only eleven were the pictures quite different (except for the pattern common to all). The following are examples of the number of times in which certain of the conditions were bilaterally present:

CONDITION	TOTAL NUMBER	NUMBER BILATERAL	PER CENT BILATERAL
Group A	70	42	60
Group B	38	24	63
Group C	68	46	64.6
Group D	24	18	75
Dorsalis pedis from perforating branch of peroneal	6	2	33.3
Perforating branch of peroneal from anterior tibial	34	14	41.1
Fourth dorsal metatarsal from posterior perforating	75	52	69.3

DISCUSSION

The extreme degree of variability in the arteries supplying the dorsum of the foot brought out in the preceding results must make it obvious that the standard textbook description falls far short of giving an entirely satisfactory concept of the arterial supply of this region. On the other hand, it would not be practical to even attempt to give a fair share of the detail included here in any relatively brief description. It is suggested, however, that a more satisfactory concept could be imparted by first describing or representing (fig. 1e) the very constant pattern of the arterial network with the understanding that any part of that network might be encountered as a vessel of significant size and that the anastomoses shown would serve as the basis for collateral circulation within the region. The description of the main groups A, B, C and D, together with their percentage of incidence could then follow logically and finally the subgroups together with a summarization of more detail if space permitted.

Although other regions have not been worked out in detail, casual observation has given no reason to believe that the dorsum of the foot is unique in having what appears to be an almost constant pattern for the arterial network supplying

it. It is therefore felt that the approach suggested above could well be used in the description of the arterial supply of many, if not all, regions. It is interesting to note in this connection that Adachi ('28) described what he called the "Grundform" of the so-called "dorsal rete" portion of the supply of the dorsum of the foot.

The fact that in almost every individual foot it was possible to find a vessel, though sometimes very fine, representing each part of the pattern of the arterial network, makes one wonder whether a network of minute vessels with this pattern might not be present as a definite stage in early development (perhaps the earliest definitive vessels arising from the primary capillary plexuses) with the resulting individual appearance depending on which channels in the network become subsequently enlarged. This, of course, is pure speculation and will require careful study to be proved correct but it would serve to explain the fact that the many variations found fell within the limits of the described pattern. In discussing the fact that vascular variations so commonly correspond to known developmental stages and represent either abnormal persistence or atrophy of channels, Poynter ('23, p. 50) makes the statement, "they are not as variable as Baader's capillary theory would imply, consequently must belong to a later stage in development, or else the early capillaries are precursors, rather than a stage in the development of the vascular tree," a thought with which the above speculation is somewhat in line.

It was pointed out on page 383 that there is no apparent agreement in the literature as to which vessel should be called the anterior perforating artery, and no one artery which seems to merit that name was found in the series of dissections. Rather there were five connections between dorsal and plantar vessels which are described under "anterior perforating arteries," two at the distal end of the intermetatarsal space, one about at the web of the toes and one in relation to the first phalanx of each of the two adjacent toes. It is suggested that it would be better to name these five

vessels the "anterior communicating arteries" and then describe the arrangements they may present in different individuals, than to attempt to choose arbitrarily the anterior perforating artery. To be logical in terminology the posterior perforating artery in each intermetatarsal space would then have to be called the "posterior communicating artery."

SUMMARY

1. The "textbook picture" of the arterial supply of the dorsum of the foot in man was present in detail in only 5.5% of the 200 feet dissected.

2. An arterial network having an almost constant pattern was demonstrated on the dorsum of the foot which can serve as a basis for a practically universally applicable description of the arterial supply of this region when information is added as to how frequently each part of that network may be of significant size, such information having been given in the description of the individual vessels making up the network.

3. The anterior medial and anterior lateral malleolar arteries were found to branch more commonly from the dorsalis pedis artery rather than from the anterior tibial artery, as usually stated.

4. No one vessel was found which seemed to merit being called "the anterior perforating artery" but in its place five vessels are described for which the name "anterior communicating arteries" is suggested.

5. A branch of the anterior tibial artery about 5 cm. above the ankle joint, a previous description of which has not been found, was present as a vessel of significant size in about half of the feet, either contributing to, or being the principal source of, the perforating branch of the peroneal artery.

6. A comparison of the findings for negro and white cadavers seems to indicate that the negro more closely approximates the "textbook picture" as far as the blood supply of the dorsum of the foot is concerned.

7. Although little exact and detailed bilateral symmetry was found, there was a rather high degree of bilateral similarity.

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BOOK REVIEW

HISTOLOGICAL STUDIES ON THE NORMAL AND THE IRRADIATED SUPRARENAL GLAND IN RABBITS. (Contribution to the subject of seasonal changes in the adrenal cortex and of the differentiation of the cortex cells.) By Olaf Torgersen. 1940. Size $4\frac{3}{4}$ in. $\times$ $7\frac{1}{4}$ in. 112 pages. 12 tables and 7 plates. Index. Paper, 1 Kommisjon hos Jacob Dybwad—1940.

This somewhat extensive but very readable treatise on the morphology of the suprarenal gland of rabbits following irradiation with roentgen rays constitutes a valuable addition to knowledge of the radiosensitivity of this endocrine gland. It is of value not only to the morphologist, interested in the anatomic implications; but to the radiologist, the physiologist and even to the clinician; for the author has given consideration to clinical data, to physiologic-chemical criteria, as well as to the pathologico-anatomical changes which the experimental procedure induced.

One feels as he reads this paper, that here is a comprehensive study carefully planned and scientifically pursued. Such factors as diet, temperature, age, season, as well as cytologic technics are rigidly controlled. A section devoted to a consideration of errors, which often complicate experimental results, indicates the caution and care with which this author has appraised his data. He points out that heterogenicity of material; dietetic variables; as well as seasonal, age, sex and temperature changes, all contribute to the diversity of results which other authors have derived from experimental studies of this sort.

Normal variability in suprarenal structure is a constant finding, too often ignored. Variations in size of cortical cells, in staining reactions, in conditions of capillaries and venous sinuses, in lipid content and chromaffin reactions, all characterize the so-called normal suprarenal organization, so that extreme caution is urged in interpreting results. This author believes that the so-called light and dark cells of the suprarenal cortex, hitherto considered probably senescent and destined for early degeneration, are really normal alterations due to seasonal variations. In these cells the doubly refracting substances, most important of which is cholesterol, are present in small amounts during winter months, but abundant in the

summer. Likewise delomorphie cells, the large cortical adipose cells of the cortex, appear normally during the months of September to April, and are especially abundant in the fall. Thus, the author has stressed the association of these regressive changes in the suprarenal gland with that period in rabbits which is characterized by a reduction or a cessation of sexual activity. To test a possible association of the light and dark cells and of these large lipoid cells of the cortex with sexual behavior, certain animals were castrated while others were given estrone injections. Light cells increased in castrates, but in estrone-injected animals they degenerated into lipoid-containing delomorphie cells. Thus the possibility is stressed that these two types of cells in the cortical zone of the suprarenal gland represent functional states associated with the elaboration of sex hormones.

The technic of irradiation which was employed is given in sufficient detail, and the manner of restricting size of areas exposed to the roentgen rays is described. With respect to the effect of irradiation on the suprarenal gland the author concluded that changes induced in either cortex or medulla, with doses ranging from 3000 to 5000 r, were but minimal. Some "vascular degeneration" of cortical cells was found; but since these same changes were observed in the cortex when other regions of the body were irradiated, the conclusions were reached that such degenerative changes are the expression of an indirect effect of the roentgen rays. The reaction of the suprarenal glands of young animals to such irradiation was very slight. Definite changes from the normal were not observed, either in the thyroid glands or in the hypophyses of irradiated animals. Sexual maturity however appeared delayed, but the complete development of the testicles was not altered by such irradiation as was employed.

GEORGE M. HIGGINS,
Mayo Foundation

REVIEWS OF APPROVED FILMS IN ANATOMY AND BIOLOGY

The following films in biology have been approved by one or more members of the Committees of the American Association of Anatomists or the American Society of Zoologists, and The Wistar Institute Committee.

Committee of the American Association of Anatomists:

Eliot R. Clark, Warren H. Lewis, Carl C. Speidel, and consultants.

Committee of the American Society of Zoologists:

Ralph Buchsbaum, Robert Chambers, Oscar W. Richards, and consultants.

Committee of The Wistar Institute of Anatomy and Biology:

Edmond J. Farris, Cranford Hutchinson, John S. Nicholas, and others as appointed.

THE HEART AND CIRCULATION

Producer: Anton J. Carlson

(400 feet, monochrome, sound)

Distributor: Erpi Films, New York

This excellent film deals with the mechanics of heart action and circulation. The natural photography includes microscopic scenes of capillary circulation, the action of the tricuspid valve and general scenes showing animal hearts in action, under various experimental conditions. Blood pressure and its relation to health is portrayed. Technical animation portrays heart and its valve action, as well as general circulation. Amplified heart sounds are excellent.

This film is recommended for undergraduate college students and medical students, and may prove of interest to the layman.

★B — E. J. FARRIS.

PINOCYTOSIS. DRINKING BY CELLS

WARREN H. LEWIS

(264 feet, monochrome, silent)

In this picture Doctor Lewis shows macrophages, in liquid tissue culture media, manifesting an extraordinary phenomenon, described by him as a result of the study of motion pictures, which he has termed "pinocytosis." The filmy fringes of the cells are in constant

activity—waving and folding over on themselves, enclosing globules of the surrounding medium. The enclosed globules can be seen to be carried toward the center of the cell where they, after persisting for varying numbers of minutes, gradually grow dim and disappear. Both normal and rat sarcoma cells are shown.

The story is exquisitely told, from every standpoint. This picture is of value both for research and teaching (see Bulletin of the Johns Hopkins Hospital, vol. 49, pp. 17-27, 1931). ★A — E. R. CLARK.

DIVIDING NORMAL ADULT RAT FIBROBLASTS IN VITRO

WARREN H. LEWIS

(160 feet, monochrome, silent)

This picture shows mitotic division in fibroblasts, as seen in "tissue culture" preparations of cells derived from adult rat tissue. A series of different mitoses is presented, which show with amazing clearness the process of cell division in typical mammalian cells. This is an exquisite picture, of great value both for research and teaching. All details are far above criticism. ★A — E. R. CLARK.

THE EYES OF SCIENCE

Producer: Bausch & Lomb Optical Company

(1200 feet, monochrome, silent)

This picture consists of three reels; the first two depict (1) certain simple principles of the behavior of light—reflection, diffraction and effect of concave and convex lenses, including a diagrammatic application to the eye; and (2) the industrial processes involved in the making of optical glass, grinding and setting of lenses, and construction of microscopes. The third reel shows some of the types of optical instruments other than microscopes which are produced by the Bausch and Lomb Optical Company, ending with several brief scenes of living biological phenomena as examples of this use of the microscope.

The film is competently executed; and the first two reels should be of interest to all users of the microscope, particularly beginners.

★D — E. R. CLARK.

★ Copy deposited in the Film Depository of The Wistar Institute, and is available to all scientists for use at The Wistar Institute.

A — Available for sale or rent by The Wistar Institute.

B — Available for sale or rent by the author or distributor.

C — Not available for sale or rent.

D — For information, communicate with the author.

E — For information, communicate with The Wistar Institute.